SELECTION FOR ENHANCED AGRONOMIC ATTRIBUTES IN *Setaria sphacelata* (SCHUMACHER) MOSS

By

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Dedicated with love to

Carina, André, Angela, and Silvano

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By

Liana Jank

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Chairman: Dr. K. H. Quesenberry
Major Department: Agronomy

There is a demand for a wider range of adapted, improved warm-season grasses in Florida because the cool winters affect the production and persistence of many existing cultivars. A breeding program was conducted in Florida to study the breeding potential and adaptation of the tropical forage grass *Setaria sphacelata* (Schumacher) Moss to these conditions. Eight populations were developed from 1997 to 1999 using half-sib family selection methodology. Six of the populations were developed in Gainesville, one in Ona and one in Quincy. In 1999 and 2000, these populations were compared in two wide-spaced and two close-spaced experiments in Gainesville. The methodology of half-sib family selection using detached culms for intermating selected populations in isolation was useful for setaria breeding, because it allowed completion of one cycle of

selection per year, it maintained variability and it avoided inbreeding. Results of population comparisons showed that selection for leaf width was successful. The width of leaves, leaf percentage, leaf:stem ratio, and whole-plant crude protein (CP) concentration were increased, while leaf dry matter yield, in vitro organic matter digestibility (IVOMD) and other measured morphological characteristics were unchanged. Stem and total dry matter yields were decreased. Selection for head number did not significantly increase the number of heads and decreased plant height, leaf width, inflorescence length, total, leaf and stem dry matter yields, leaf percentage and leaf:stem ratio in one or both cycles. Seed production, CP and IVOMD were not affected. Selection for regrowth after grazing reduced plant height, inflorescence length and seed production. Selection for frost tolerance was successful in enhancing winter survival at Gainesville and reduced canopy and inflorescence height and leaf dry matter yield in one or two cycles. It did not affect leaf width, inflorescence length, head number, total or stem dry matter production or leaf:stem ratio. The germplasm of *S. sphacelata* was plastic enough that changes in morphological and agronomic traits due to selection and breeding were achieved. Setaria has promise for use as a forage plant in Florida, but concerns regarding pest susceptibility and winter persistence remain to be resolved before this species may be commercialized in the state.

CHAPTER 1
INTRODUCTION

In the southern USA, cow-calf production is the major beef enterprise (Wilson and Watson, 1985), and at least 80% of the weaned calves are sold to producers in the Great Plains and the Midwest. The efficiency of these livestock systems is, however, not very high. For example, live-weight gains of only 0.45 kg $head^{-1}$ day^{-1} were obtained from bahiagrass pastures (*Paspalum notatum* Fluegge) (Sollenberger et al, 1988). Bahiagrass is the most widely grown cultivated species in Florida, and covers approximately 1 million ha (Chambliss and Sollenberger, 1991). Other species, such as limpograss [*Hemarthria altissima* (Poir.) Stapf & Hubbard] and digitgrass (*Digitaria eriantha* Steud.) are used to a variable extent, but present problems of low forage N concentration and poor cold tolerance, respectively. The cold winter temperatures in Florida limit the use of digitgrasses and stargrasses (*Cynodon nlemfuensis* Vanderyst) for animal production. Improved warm-season grasses that are productive and persistent under the existing environmental constraints, and provide high quality grazed forage, have the potential to increase the efficiency of beef cow-calf systems in the South, especially in Florida (Hoveland, 1986).

The tropical forage grass *Setaria sphacelata* (Schumacher) Moss is used as a pasture grass for grazing of dairy and beef cattle throughout the world and also as cut-and-carry, silage or hay. It is high yielding (Drudi and Braga, 1990; Singh et al., 1995), persistent (Gibson and Andrews, 1985), and palatable (Ghisi et al., 1994). It presents high

crude protein (Bufarah et al., 1982; Alvim et al., 1986), superior seasonal growth distribution (Pedreira and Mattos, 1981) and adequate animal production (Hillesheim et al., 1987; Alvim et al., 1993). For milk production, *S. anceps* (*S. sphacelata* var. *sericea*) 'Narok' was superior to *H. altissima* in southern Brazil (Martinez, 1993). Meat production on *S. sphacelata* 'Kazungula' was higher than on *Brachiaria brizantha* (Hochst. Ex A. Rich.) Stapf pastures in Mexico (Partida et al., 1983). Recent evaluations have shown that *S. sphacelata* may have potential for use under Florida conditions.

A population of *S. sphacelata* was available at the Agronomy Forage Research Unit (AFRU) adjacent to the Dairy Research Unit of the University of Florida, at Gainesville, Florida. The population was a bulk of seeds of Narok, Kazungula, 'Solander' and roadside plants harvested by Dr. P. Mislevy at the Ona Research and Education Center of the University of Florida at Ona. This population was used as a base population for the breeding program conducted from 1996 to 2000. Breeding consisted of the development of improved populations for morphological characteristics and for frost tolerance (Chapter 3). Two cycles of selection for increased seed head number, one cycle for increased leaf width and one for grazing tolerance were evaluated for selection effects on various morphological and agronomic traits (Chapter 4). Populations from three cycles of selection for frost tolerance at the AFRU and one cycle at Quincy were evaluated for selection effects on various morphological and agronomic traits (Chapter 5).

CHAPTER 2
LITERATURE REVIEW

Origin and Adaptation of *S. sphacelata*

The genus *Setaria* P. Beauv. belongs to the Subfamily Panicoideae, tribe Paniceae, and includes about 100 annual and perennial species extending from the tropics into the subtropics (Clayton and Renvoize, 1982). The name "Setaria" comes from the latin "seta" meaning bristle, and species in the genus are characterized by having one or more bristles immediately below some or all spikelets. These bristles are considered to be sterile panicle branches. In some species they subtend the spikelets throughout the inflorescences whereas in other species they only subtend terminal spikelets. They persist on the axis when the spikelets fall off (Clayton and Renvoize, 1982).

The main forage species is *S. sphacelata* and throughout this document the common name setaria will be used for this species. It originated from Africa and is naturally distributed from the Sudan to subtropical South Africa and from the Ivory Coast to Ethiopia and Yemen (Clayton and Renvoize 1982). However, the main forage varieties (var. *sericea* and var. *splendida*) originate from East and Central Africa.

The species *S. sphacelata* occurs naturally from sea level to 3300-m altitude (Clayton and Renvoize, 1982) and on a variety of soil types, from waterlogged and swampy clays to infertile sandy and granitic soils (Rattray, 1960). In East Africa it may be found at altitudes above 2,100 m and rainfall zones between 750 and 2,000 mm per

annum and along stream banks, forest margins and wet grasslands subject to occasional flooding. Most plant collections were from slightly acidic soils (Jones, undated).

The species has been recommended for use in tropical and subtropical countries with a minimum yearly rainfall of 750 mm, or 580 mm on very fertile soils (Botha, 1948). However, it grows better in wetter areas with no prolonged dry season (Rattray, 1960), as in the wet zone of Sri Lanka (Assefa and Premaratne, 1990), on dairy farms in the humid tropics of Costa Rica (Sanchez et al., 1998), and in periodically waterlogged soils in Brazil (Alvim et al., 1993) and in Thailand (Hare et al., 1999). Animal production on setaria pastures can be limited by low rainfall (Jones and Evans, 1989; Jones and Jones, 1989).

The latitudinal limits to growth are about 30° North and South (Russell and Webb, 1976), although in New Zealand, it has been recommended at latitudes as far south as 37° (Davies and Forde, 1991). In north Guangdong, China, Kazungula is recommended for regions with around 14 frosts per annum and an absolute minimum temperature of - 4.6°C, where other grasses, such as guineagrass (*Panicum maximum* Jacq.) and rhodesgrass (*Chloris gayana* Kunth) have limited potential due to poor frost tolerance (Michalk and Huang, 1994). In China, Kazungula tolerated -8.2°C in winter (Wu et al., 1986). Kazungula also tolerated 41.3°C in summer along with drought, waterlogging and soil pH of 4.5.

The species *S. sphacelata* is adapted to fertile loam to sandy soils (Shankarnarayan and Dabadghao, 1972), but not to heavy clays. Nineteen grasses were evaluated in China on sandy and on fertile loam soils and Kazungula setaria only performed well on loam soils (Michalk et al., 1993). In seasonally waterlogged soils in

northeast Thailand, 'Splenda' setaria grew well but was less productive than *Paspalum atratum* Swallen (Hare et al., 1999). Setaria cultivars grew well in pastures on sandy gleyed podsolics derived from sandstones at Beerwah, southeast Queensland (Hacker and Evans, 1992), providing adequate fertilizer was supplied. Setaria is not adapted to saline soils, and in a glasshouse experiment 'Nandi' setaria proved to be the least tolerant of 10 grass species tested (Russell, 1976).

Genetics of *S. sphacelata*

The species *S. sphacelata* is a polyploid complex ranging from diploid to decaploid that at higher levels of polyploidy may readily be inter-crossed (Hacker, 1968b). The basic chromosome number is x=9. The most frequent levels of polyploidy are diploid (2n = 18), tetraploid (2n = 36) and hexaploid (2n = 54). However, octoploids (2n = 72) and decaploids (2n = 90) also occur (Hacker, 1966). Octaploids and decaploids apparently come from the margin of the species distribution, from western and southern Africa, respectively. There are records of some pentaploids (2n = 45) in nurseries, but it is not known whether pentaploids occur naturally in the wild. The species is largely cross-pollinating and apomixis has not been recorded. However, self-pollination may also occur (Hacker, 1967).

Of the released cultivars, Nandi is diploid (Hacker, 1966) and Kazungula, Narok, Solander and Splenda are tetraploid (Hacker, 1966; Oram, 1986). Reported chromosome numbers in *S. sphacelata* var. *splendida* are 2n = 4X=36 (Hacker, 1968a), 2n = 5X=45 (Hacker, 1966) or (2n=7X=63) (Moffett and Hurcombe, 1949). The vegetatively propagated cultivar of *S. sphacelata* var. *splendida* known as Lampung, widely grown in southeast Asia (Horne and Stür, 1999), is thought to be CPI 15899, a tetraploid. Vigorous

and high-yielding morphotypes are frequently tetraploid while those exhibiting some degree of frost tolerance are usually hexaploid. Plants that exhibited abundant rhizomes had high chromosome numbers of 72 and 90, while at lower levels of ploidy, rhizome development was limited (Hacker, 1966).

Hacker (1968b) found that there is no cytological barrier for gene exchange among the different varieties. Crosses between different varieties at the same ploidy level (e.g., var. *torta* x var. *sericea* and var. *sericea* x var. *splendida*) resulted in plants with a high degree of meiotic pairing, indicative of chromosome homology (Hacker, 1968b). However, since the limits of the different botanical varieties are undefined due to a lack of chromosome differentiation, the complex is at an incomplete stage of speciation (Hacker, 1968b).

At least some of the genomes of the complex are homologous. Pentaploids derived from 4x X 6x crosses, septaploids from 6x X 8x crosses and nonaploids from 8x X 10x crosses all have predominantly bivalent pairing, with infrequent trivalents and 1 to 9 univalents; almost all have some fertility, producing seed. Based on meiotic analyses of these interploidy hybrids in the *S. sphacelata* complex, Hacker (1968b) concluded that the complex is an autotetraploid series. He further stated that the inhibition of pairing between homologous chromosomes in these autopolyploids could be under genetic control.

Extra chromosomes, probably *B*-chromosomes, occur in some hexaploid introductions. It is hypothesized that these chromosomes have arisen very early in the evolution of the species complex (Hacker, 1966).

Breeding and Cultivar Development in *S. sphacelata*

Early breeding focused on *S. sphacelata* var. *sericea*. Several breeding programs were attempted and/or are underway. The main traits that were the focus of breeding programs were increased seed production and increased frost tolerance (van Wijk, 1980; Hacker, 1991a; 1991b; Hacker and Cuany, 1997; Tomei et al., 1998). A further selection criterion in all breeding programs has been yield.

The breeding history of the species traces back to Kenya. Nandi was the first cultivar to be selected. It originated in Kenya and was selected in 1947 for use as a pasture grass. It was introduced into Australia in 1961, soon becoming popular in humid areas of southeast Queensland. After its release, it was further selected in Kenya for greater uniformity and general good performance (Bogdan, 1965). The selected cultivar was released as 'Nandi Mark 2'. It was later flowering and produced less seed than Nandi. Mark 2 eventually replaced Nandi, but has retained the name Nandi in commerce.

Kazungula is of southern African origin and was introduced into Australia in 1962. Kazungula had previously been developed in South Africa for grazing and hay production (Oram, 1990), although Oram (1990) states that the parental ecotype was native to Zambia. The name was applied earlier to an ecotype that was collected at the border of Botswana and Zimbabwe and that was studied in South Africa (Gildenhuys, 1951). This cultivar was developed for use as fodder grass as silage, hay or green chop. Nandi and Kazungula were named after their collection site (Hacker and Jones, 1969). Plants of Nandi and Kazungula are tolerant of light frosts but aboveground parts are totally killed (Hacker, 1991a).

In Brisbane, Australia, introductions were assembled from East, South, Central and West Africa, partly from overseas collections and partly from the 1963 collection by

R.J. Jones, focussing on genotypes tolerant of frost (Hacker, 1966). The range of distribution of the complex in Kenya includes mountainous areas in the Aberdares Range where night temperatures fall below 0°C, and introductions from this region of Kenya were shown to exhibit frost tolerance (Hacker, 1972).

Narok was the first wintergreen cultivar to be released (Oram, 1990). The parental introduction of Narok, CPI 33452, originated from an altitude of 2200 m in the Aberdares Range of Kenya. It was named after a small river, the Uaso Narok, close to where the parent introduction was collected. Two cycles of selection for winter yield and winter greenness were undertaken and the cultivar Narok was registered in 1969 (Hacker, 1972). Despite having a low frost tolerance compared to other frost tolerant introductions from the Aberdares Range, it had high winter-spring productivity and could withstand frosts of -3°C without showing leaf damage. Its seed production was low however, due to low tiller fertility in stands more than one year old (Hacker, 1972). Narok is widely grown from New South Wales to the Atherton Tableland in north Queensland, but has to some extent been replaced by Solander. In more tropical sites at low altitudes, Kazungula tends to be the preferred cultivar (Jank and Hacker, in press).

The *S. sphacelata* var. *splendida* was collected as early as 1938 in East, Central and South Africa on swamp margins (Anon, 1945). It is more robust than var. *sericea*, having a higher number of culm nodes, greater culm diameter, and larger leaf width and height. It is also later flowering. The introduction CPI 15899 exhibits the var. *splendida* morphotype and was studied extensively as an introduction and was used as a parent in the breeding programs in Australia. Cattle weight gains from this introduction were reported to be higher than from three cultivars of var. *sericea* (Evans and Hacker, 1992a).

However, all accessions of var. *splendida* tested in Queensland were almost sterile. Variety *splendida* Lampung, thought to be CPI 15899, is widely planted in southeast Asia, where it is vegetatively propagated (Horne and Stür, 1999). The introduction CPI 15899 was crossed with several accessions of the Kazungula morphotype of var. *sericea*, and selection for seed fertility and for var. *splendida* phenotype was carried out in generations F2 to F4. Two of the best populations were combined and registered as 'Splenda', which has the var. *splendida* morphotype (Hacker, 1985). In the literature there are many reports of a "*Setaria splendida*", "var. *splendida*" or "cv. Splendida", with no reference as to which cultivar or introduction they are. From here on, these cases will be referred to as splendida.

Further crosses were made between tetraploid accessions of var. *sericea* from high altitudes, lowland Kazungula types, and var. *splendida* CPI 15899. Selection was carried out in F2 and F3 for frost tolerance and winter yield. Three F4 populations were then compared with commercial varieties in a sward, and selection was based on seed production. Derivatives of the best populations were released as Solander in 1984 (Hacker, 1985). Solander produced twice as much seed as Narok due to double the tiller fertility and number of heads per unit area. However, seed yields were still below those of Kazungula (Hacker, 1985).

Other setaria strains selected include Bua River and du Troitskaal. Bua River also was developed for use as silage, hay or green chop. Du Troitskaal ($2n = 90$) is a drought resistant, strongly rhizomatous strain that has never been grown commercially outside of South Africa (Gildenhuys, 1951; Hacker and Jones, 1969). An ecotype under the name Gomoto Mongolelo River (also spelled Gomoti River/Mogogelo) from Botswana

(Gildenhuys, 1951) has been listed as useful for hay and silage in South Africa (Skerman and Riveros, 1990) and has been tested in Brazil (Rocha et al., 1994). There are reports of a cv. Morado in Costa Rica (Villareal et al., 1994). These ecotypes are however, apparently not grown commercially.

Success in breeding cross-pollinating species, such as *S. sphacelata,* can be expected when the traits to be selected exhibit additive genetic variance with a relatively high level of narrow-sense heritability. Useful levels of variation within the complex were found in many important traits, including yield and its seasonal distribution, digestibility, seed yield, frost tolerance and mineral, Na and oxalate concentrations (Hacker, 1974b; Hacker and Bray, 1981; Bray and Hacker, 1981). However, breeding programs are necessarily long term, with no guarantees of improved animal production from the resulting new cultivars, and in most countries opportunities for funding such programs are limited or non existent (Jank and Hacker, in press).

High heritabilities were found for leaf width (Hacker, 1987), inflorescence length and tiller fertility (Hacker and Cuany, 1997), suggesting that these traits can be easily manipulated by the breeder. Broad leaves may increase sward density and thus may increase short term intake by cattle. Inflorescence length and tiller fertility may increase seed production. Vegetative leaf width was positively correlated with flower head number and with dry weight. Flag leaf width was controlled by different genes than those controlling vegetative leaf width, and was positively correlated to days to flowering and negatively correlated with flower head number. Selection for increased dry matter yield should result in earlier flowering plants with shorter inflorescences (Hacker and Bray,

1981). Natural selection within Nandi that was heavily grazed for several years resulted in earlier flowering plants with higher head number (Hacker, 1987).

Setaria is comparable in digestibility to other tropical forages, which are generally less digestible than their temperate counterparts. The low quality of setaria in comparison with temperate grasses results from low leaf digestibility, not low digestibility of stem or sheath (Hacker, 1971). Therefore, for the production of higher quality, selection for leaf digestibility is necessary (Bray and Hacker, 1981). Selection for digestibility may be conducted successfully in any season, locality or growth stage, as superior accessions are generally superior over a wide range of environments. However, as there is a wider range of variation in IVOMD in summer than in winter due to higher growth of plants, it is preferable that selection be carried out in summer (Hacker and Minson, 1972). Selection for whole-plant digestibility should result in increased leaf and stem digestibility if selection is made within a population of the same ploidy level (Hacker, 1974a). Among different ploidy levels, hexaploid var. *aurea* introductions had 3.8 to 4.3 units higher leaf digestibility and lower stem digestibility than tetraploid var. *sericea* introductions, despite whole-plant digestibility being similar. The lower stem digestibility of hexaploids was due to more mature stems because of earlier flowering. In tetraploids, flowering was negatively correlated with leaf percentage (Hacker, 1974a). In vitro digestibility, however, was correlated negatively with dry matter yield (van, 1980), and only weakly negative in the work of Bray and Hacker (1981), suggesting that selection for high yield should have only minimal effects on depression of digestibility.

Production and Quality of *S. sphacelata*

The most widely sown cultivar is Kazungula. In tropical low altitude regions, Kazungula is more drought tolerant than other cultivars. It is very productive in summer, but this is associated with a high production of stem, which is also responsible for its high seed production in comparison to other cultivars. It has acceptable nutritive value, is palatable for cattle and has annual yields higher than 90 t ha^{-1} fresh matter (Jank and Hacker, in press). Of 38 grass species in Brazil, dry matter yield was greatest in Kazungula and crude protein concentration (146 g kg^{-1}) was also high (Almeida and Flaresso, 1991). In comparison to seven other warm-season grasses, it showed the greatest potential for forage production during the dry period in the southern region of Minas Gerais, Brazil (Botrel et al., 1999). Although it is considered the most drought tolerant of the setaria cultivars, it is less tolerant than other warm-season species such as *Cenchrus ciliaris* L. and *Urochloa mosambicensis* (Hack.) Dandy (Jank and Hacker, in press). In a cafeteria-type experiment, Kazungula produced the best ground cover (72%), one of the highest yields and crude protein percentages, and a high acceptability, mainly in the rainy season (Botrel et al., 1987). It was recommended for degraded areas due to its great natural reseeding.

In contrast to Kazungula, Nandi and Narok are recommended for subtropical areas. They are leafier than Kazungula in summer, and in winter Narok and Solander retain green leaves after light frosts. In the subtropics, setaria grows from spring through autumn, with peak production in summer, depending on rainfall. In a comparison of Narok, 'Coastcross-1' bermudagrass and 'Roxinha' limpograss in Parana, Brazil, Narok gave the highest milk fat, crude protein, in vitro dry matter digestibility and yield in the summer, while Coastcross-1 gave the longest period of grazing and highest dry matter

availability in the autumn (Martinez, 1993). In another experiment in Santa Catarina, subtropical southern Brazil, Nandi and splendida showed a good annual distribution of production: 60% of their annual dry matter production was produced in spring and autumn, periods of high forage deficit. Splendida produced well in autumn (33%), while Nandi produced 45% of its yield in spring. Narok and Kazungula produced 46% of their yield in spring and autumn combined (Rocha et al., 1994).

In Indonesia, strip grazing of splendida pasture (6.25 cows ha^{-1}) was profitable whereas stall feeding of guineagrass resulted in economic loss (Soetrisno et al., 1994). Cultivar Splenda is recommended as a cut-and-carry fodder grass for stalled animals, or for grazing, in southeast Asia and the Pacific islands (Davidson, 1988). In dairy farms in Zaire, silage of splendida is recommended for the dry season (Dupont et al., 1995). Ensiling setaria in plastic silos is a good practical option for the smallholder farmer in Indonesia (Sunarso et al., 1994).

The cultivars Narok, Nandi and Kazungula were compared in a field trial in a subtropical climate in India. Dry matter yields, crude protein yields, seed yields and weight of 1000 seeds were highest for Nandi, followed by Kazungula, then Narok. There were no differences among the three cultivars for tussock diameter, number of vegetative tillers, number of fertile tillers per plant or spike length. Kazungula showed higher root production, root volume, number of roots per tussock and soil binding capacity (Dwivedi et al., 1991).

In subtropical Queensland, Australia, although there are reports of steer gains being similar for cultivars Narok, Kazungula and var. *splendida* CPI 15899 (Jones and Evans, 1989), other reports show the superiority of one cultivar over another (Evans and

Hacker, 1992a; 1992b). Liveweight gains with 4 to 6 steers ha^{-1} and digestibility were higher for Narok followed by CPI 15899, then Nandi and Kazungula. Narok produced 24 kg $steer^{-1}$ yr^{-1} more than splendida (Evans and Hacker, 1992a; 1992b).

In comparison with the productive *P. purpureum* Schum cv. Napier under dairy cattle grazing in the subtropical south of Brazil, Kazungula had a higher dry matter availability, milk production cow^{-1} day^{-1}, and equal fat percentage and cow weight, but much inferior carrying capacity. Kazungula carried 1.1 cows ha^{-1} while Napier carried 4.2 cows ha^{-1} (Olivo et al., 1992).

Good protein levels were reported in young growth of *S. sphacelata* (Almeida and Flaresso, 1991; Wu et al., 1992; Alvim et al., 1993), however, protein decreases very rapidly with maturity and flowering (Perera et al., 1990; Singh and Pradhan, 1995). Crude protein concentration in India decreased from 130 to 95 to 63 g kg^{-1} when grown for 20, 40 and 60 d, respectively (Singh and Pradhan, 1995). In another experiment, concentrations were 157 g kg^{-1} before flowering and 54 and 64 g kg^{-1} at mid- and full-flowering, respectively. These protein values at flowering were considered to be insufficient for the maintenance requirements of animals (Perera et al., 1990). The N balance in setaria was found to be negative and N quality poor, therefore Singh and Narang (1992b) considered that setaria should not be offered as a sole feed to animals. In contrast, Bray and Hacker (1981) found that in a range of genetic populations of setaria studied over a 3-yr period, harvested at 6-wk intervals and fertilized at 50 kg ha^{-1} after each cut, N concentration was almost always above 15 g kg^{-1} (94 g crude protein kg^{-1}) and frequently above 20 g kg^{-1} (125 g crude protein kg^{-1}).

Digestibility values in setaria are comparable with other tropical grasses, but at least one experiment showed a digestibility of 640 g kg^{-1} for setaria before flowering, while guineagrass showed 760 g kg^{-1} (Perera et al., 1990). In a comparison with other forages, two introductions of *S. sphacelata* var. *splendida* had a 60 g kg^{-1} higher digestibility than *P. maximum* or *Pennisetum clandestinum* Hochst. Ex Chiov. (Hacker and Minson, 1972). When compared to the temperate species timothy *Phleum pratense* L., the lower quality of setaria resulted from lower leaf digestibility, and not stem or leaf sheath digestibilities (Hacker, 1971). Leaf digestibility of setaria varied from 575 g kg^{-1} for the top flag leaf to 48 g kg^{-1} in the 6th leaf. In another experiment, stem digestibility (654 and 614 g kg^{-1}) was only slightly lower than leaf digestibility (673 and 643 g kg^{-1}) for two harvest dates (Hacker, 1974a). In Australia, whole-plant digestibilities up to 710 g kg^{-1} were reported for 4-wk regrowth harvested in mid-summer (Hacker, 1974a). Winter values tended to be higher than summer values (Hacker and Minson, 1972).

As with other tropical grasses, digestibility in setaria decreases with flowering. In India, digestibility values were 640, 478 and 452 g kg^{-1} at pre-, mid- and full-flowering, respectively (Perera et al., 1990). Moss and Murray (1992) recorded a drop in digestibility from 660 g kg^{-1} pre-flowering to 400 g kg^{-1} with flowering. However, rapid lignification of culms is likely to result in their becoming less digestible than leaves as they age. Leaf:stem ratio in setaria (cultivar unspecified) was also low, 1.1 before flowering, whereas in guineagrass it was 3.0 (Perera et al., 1990). In Australia, however, leaf:stem ratios varied from 0.94 to 2.08 for four different cultivars (Hacker and Evans, 1992). Leaf:stem ratio in setaria varies widely with season, cultivar and stage of growth, with Narok usually being much leafier than other cultivars, especially Kazungula.

The feeding value of setaria as a pasture grass depends on soil fertility, fertilizer applied, stage of growth and opportunity for steers to select a high-quality diet. In a N-fertilized trial in Australia in a drier-than-average year, plucked leaf samples, comparable with steers' diet, had in vitro digestibility of 680 to 760 g kg^{-1} during the summer, depending on cultivar (Evans and Hacker, 1992b).

As with other forages, fertilization with N increased crude protein and ether extract concentration in Kazungula, but decreased the crude fiber and ash concentration. With a longer growth period, crude fiber increased while crude protein, ether extract and ash concentration decreased (Zanetti et al., 1992).

Although setaria has many positive attributes as a tropical forage, it also has some negative qualities. Kazungula has been reported to be susceptible to the spittlebug species *Zulia entreriana* in Brazil (Nilakhe et al., 1985), to the fungal disease *Pyricularia* sp. (Bogdan, 1977) and to *Sphacelotheca* sp., *Fusarium ninale* var. *majus* and *Tilletia echinosperma* (setaria bunt) (Bogdan, 1977) in the inflorescences in Africa.

Lower intake in cv. Kazungula and Narok as compared to *P. clandestinum* (kikuyo) and *Avena sativa* forages were associated with lower crude protein, low nitrogen intake and digestibility of crude protein, and a high NDF content (Singh and Narang, 1992b). Physical limitations in cell wall constituents, digestibility and degradation also limited intake (Singh and Narang, 1992a). In comparison with other warm-season grasses, setaria Kazungula had 8.5% higher lignin content and higher cellulose content (Ford and Elliott, 1987), 3 to 9% higher crude fiber content in cv. Nandi (Singh et al., 1995) and 17% higher indigestible fraction in Kazungula and Narok (Singh and Narang,

1992b). This resulted in slower disappearance by fermentation and consequently higher rumen fill (Singh and Narang, 1992b).

Animals grazing setaria may also experience problems from oxalate poisoning, but deleterious effects are uncommon in cattle, and generally only occur when cattle that have not previously been exposed to the grass are offered fertilized setaria as their sole source of feed. However, oxalate poisoning is more common in monogastric animal such as horses (Walthall and McKenzie, 1976). This condition is not specific to setaria, but also occurs widely with buffel grass, *Cenchrus ciliaris* L.

Oxalate accumulation was found to be associated with area of origin of the introductions. South African accessions, var. *splendida* and Kazungula had high oxalate concentrations. Accessions from Kenya, such as Nandi and the frost tolerant accessions of var. *sericea*, and *S. trinervia* (now classified as var. *aurea*) had low concentrations (Hacker, 1974b).

Breeding for Increased Seed Yield

Increased seed production in setaria has been the objective of many breeding programs (van Wijk, 1980; Hacker, 1991a; 1991b; Hacker and Cuany, 1997; Tomei et al., 1998). Potential for improving seed yield in setaria is still high and there are large differences in components of seed production among genotypes within cultivars (Hacker and Cuany, 1997). Increase in seed production in Narok and Solander can be expected through improved synchrony of flowering through phenotypic selection, and through selection for tiller fertility within genotypes with a similar initial flowering date. Increased seed production in Kazungula can be expected through selection of total number of tillers plant^{-1}, because most of the tillers are fertile. In general, seed production

could be improved by selecting for a high number of inflorescences in genotypes flowering early to mid-season (Hacker and Cuany, 1997) and for greater uniformity in ripening (Hacker and Jones, 1971).

Seed production in setaria is low compared to most other warm-season forage grasses (Hacker and Loch, 1999), although cultivars differ, with Kazungula having comparatively high seed yields. Seed yields of setaria in Queensland were the lowest of the grass cultivars discussed by Humphreys and Riveros (1986). In India, Nandi setaria produced 70 kg seed ha^{-1}, while four *P. maximum* cultivars produced from 150 to 530 kg ha^{-1} (Singh et al., 1995). Low percentage of fertile tillers in Nandi, prolonged flowering, uneven ripening within the inflorescence and a high percentage of shattered seeds at harvest all contribute to the low seed yields. Fukuyama and Ito (1997) found that the ratio between percentage of heading tillers and total tiller number in Narok was considerably lower than those in Kazungula or two *P. maximum* cultivars, a result supporting the study of Hacker and Cuany (1997). Hacker and Jones (1971) found that 93% of the potential seed was lost, either due to failure of seed set, immaturity at harvest or shattering before harvest.

Shuan and Jun-Quan (1989) recorded that most tiller emergence in Narok occurred within the first 15 d after fertilizer application. The fertilizer was applied just after the beginning of the wet season, when the grass had started to regrow. Maximum tillering occurred around 16 to 30 d, and maximum heading around 60 d after fertilization. After heading, heads matured in 30 d, and started shedding 10 to 20 d later. Differences among cultivars exist, but in general, best harvest times were from 38 to 44 d after initial head emergence (IHE), considering IHE when 10 inflorescences m^{-2} had

emerged. Thus, optimal harvest time is when shattering has already commenced. In Brazil, the best time to harvest Kazungula seeds was 29 to 36 d after IHE, considering quantity of seed produced, purity and germination (Junqueira et al., 1985b). When considering yield of pure germinable seeds alone, 35 d after IHE was the best harvest time (Zago et al., 1984). Conde and Garcia (1991), however, found that highest seed germination of Kazungula occurred when seeds were harvested from 38 to 44 d after IHE, and when stored at room temperature for 4 mo. Before this storage period, seeds exhibit dormancy and after this time, they begin to senesce. Physiological maturity in Kazungula seeds was reached 38 d after IHE, thus seeds should be harvested 32 to 38 d after IHE (Conde and Garcia, 1984).

Seed production in setaria usually is increased by N application, which increases flowering tiller number per unit area (Hacker and Jones, 1971; Junqueira et al., 1985a; Cruz et al., 1989; Hacker, 1994; Dwivedi et al., 1999). Seed yield is highly correlated with head density per unit land area (r=0.90) (Shuan and Jun-Quan, 1989). The plant introduction from which Narok was derived still increased seed yield at the highest N fertility level of 336 kg ha^{-1} yr^{-1}, the effect of N on seed yield and seed head number being linear (Hacker and Jones, 1971). Junqueira et al. (1985b) suggested a value between 0 and 100 kg ha^{-1} would be the most economical. However, in some reports, seed yields were not increased with N (Stillman and Tapsall, 1976; Junqueira et al., 1985b) but seed germination (Stillman and Tapsall, 1976) and fresh and dry matter yields (Junqueira et al., 1985b) were increased. The general trend regarding effects of N fertilization on seed yield appears to be an increase with increasing levels of applied N until a level where diminishing returns occur.

Nitrogen also increased the number of vegetative and reproductive tillers in Kazungula (Cruz et al., 1989), and plant height, fertile tillers per plant, spike length, seed yield, 100-seed weight and germination in Nandi (Dwivedi et al., 1999). Response to N, however, resulted mainly from the increased number of fertile tillers (Dwivedi et al., 1999). Applying half of the N at the beginning of the growing season and half after 2 mo did not increase seed yields of Narok (Hacker and Jones, 1971).

Comparing the different cultivars, Kazungula has higher pure seed yields, Narok the lowest, and Nandi and Solander have intermediate values (Hacker, 1991b; Hacker and Cuany, 1997). In Australia, Kazungula, Solander, Nandi and Narok had seed yields averaging 293, 100, 89 and 57 kg ha^{-1} yr^{-1}, respectively (Hacker 1991b). With the application of 100 kg N ha^{-1}, yields of Solander and Narok were 68 and 31 kg ha^{-1} at the first harvest of a 3-yr sequence, respectively, whereas seed yields were 39 and 20 kg ha^{-1} when only 25 kg N ha^{-1} was applied (Hacker, 1994). Narok had higher germination percentages, which was related to less dormancy. This was attributed to Narok having been selected from an accession from the Aberdares Mountains in Kenya where there is no pronounced dry season (Hacker, 1994). However, for both cultivars, seed production fell drastically over the course of the experiment. Kazungula also produced more seed than Solander, followed by Nandi and lastly Narok in another experiment (Hacker and Cuany, 1997). Intra-cultivar variability in flowering and seed production characteristics of each cultivar were discussed by Hacker and Cuany (1997), who showed a wide genetic variation within Narok and Nandi in heading date, with early-heading genotypes being first to flower in both crops in the growing season. Broad sense heritability for seed

yields averaged 0.68, indicating good opportunities for selecting cultivars with higher levels of seed production.

Breeding for Frost Tolerance

Variability within the complex for frost tolerance exists and was shown to be related to altitude of origin (Hacker et al., 1974). Within the frost tolerant introductions, the most frost tolerant were hexaploids while the least tolerant were tetraploids, and there was a negative relationship with vigor. Some frost-tolerant introductions did not persist, probably due to a susceptibility to short cutting height (Hacker, 1972). Frost tolerance also showed no association with alcohol soluble carbohydrates (Hacker et al., 1974).

In the breeding program in Queensland, Narok and Solander were bred for frost tolerance (Hacker, 1972; Hacker et al., 1974; Hacker, 1985; Oram, 1990; Hacker, 1991a). Narok was bred from an introduction collected at 2200-m altitude at the Aberdares Region of Kenya, followed by two cycles of selection for winter yield and greenness (Hacker, 1972). Solander was bred from high altitude tetraploid accessions of the *var. sericea* type and lowland accessions of the Kazungula type and one introduction of *var. splendida* (Hacker, 1985). Selection for winter yield and frost tolerance were done in F2 and F3.

Different cultivars have different tolerances to low temperatures. Nandi is less tolerant of temperatures of -4°C than splendida or Kazungula (Hacker and Jones, 1969), although plants of both survive brief periods with considerably lower temperatures. Selection for winter yield could result in a decrease in mineral concentrations in the summer, since the genetic correlation of N, K, Ca and Mg concentrations with yield was negative (Bray and Hacker, 1981).

Narok survived three consecutive nights at -3.5°C in simulated frosting experiments (Davies and Forde, 1991). Mortality resulted from frost followed by a period of chilling. Three nights at -3.5°C followed by 2 to 6 wks of chilling at 13°C day and 5°C night temperatures resulted in 40 to 50% death of the Narok plants. No plants survived after frost and 105 d of chilling (Davies and Forde, 1991).

Despite Narok having been selected for frost tolerance, its frost tolerance does not compare with that of *Paspalum dilatatum* Poiret (Hacker et al., 1974). However, Setaria withstands light frost to an extent other warm-season grasses are susceptible. In China, it is recommended for regions which have around 14 frosts yr^{-1} and minimum temperatures of -4°C, where other grasses such as guineagrass and rhodesgrass are limited due to poor frost tolerance (Michalk and Huang, 1994). In China, Kazungula tolerated -8.2°C in winter (Wu et al., 1986).

Substantial variation in frost tolerance among cultivars of bermudagrass (*C. dactylon*) has been found, and may also be used for breeding improvement (Anderson and Taliaferro, 1995). Genetic variability for winter hardiness was also found for limpograss germplasm, and this variability could be used to expand the production range of the species through breeding (Oakes, 1989).

Recurrent Restricted Phenotypic Selection (RRPS) Breeding

The RRPS breeding is a methodology proposed by Dr. G. Burton to increase the yields of Pensacola bahiagrass (*Paspalum notatum* var. *saurae* Parodi) (Burton, 1982). It is a modification of the conventional mass selection, where eight restrictions were imposed and the efficiency has increased four-fold. In RRPS, ca. 20% of selected plants are intermated with each other in a polycross in isolation. Three complete culms, with

some stolon attached, of each selection that will flower the next day are harvested, placed in a container of water, covered with a paper tent and shaken several times each morning for interpollination. With this procedure there is control of both male and female progenitors and the efficiency of regular mass selection is doubled (Burton, 1982). Also by this procedure, the number of years required per cycle is reduced from three to one. This methodology also avoids inbreeding and decrease in variability since one-fifth of the initial population is maintained in the polycross. For diploids, a minimum of 100 or more plants should be selected from each cycle.

Hacker and Cowper (1984) suggested a metal crossing chamber for the breeding of cross-pollinated grasses. Two pots to be crossed are put in the chamber and different size plants can be crossed. Pollination is favored by wind blown into the chamber from a forge blower. This procedure favors the circulation of pollen so plants with low fertility successfully produce seeds, eliminates molding because of an improved circulation of air, and avoids the breaking of culms as they elongate since the framework is elevated as necessary. Although this chamber is very useful in particular cases, the RRPS methodology is simpler and permits a greater number of selections to be crossed thus reducing inbreeding. With the RRPS, different size plants may also be crossed and because a great number of inflorescences are crossed and tied together, their culms support each other and breaking of culms is minimal. By shaking several times in the morning, pollen is dispersed, trapped in the bag and distributed evenly across the flowers under the bag. This method is simple, cheap and promising in the breeding of other cross-pollinated tropical grasses. Some of the restrictions to regular mass selection suggested

by Burton (1982) such as use of detached culms and intermating under paper bags may also be applied to improve half-sib progeny testing breeding methods.

Summary

Based on the review of literature it can be concluded that variability for many traits exists in the *S. sphacelata* complex and progress through breeding and selection is possible. The breeding work in Australia successfully improved and incorporated into existing cultivars traits such as leafiness, improved seed production and tolerance to frost.

The breeding work with Setaria in Florida had three objectives:

1. To evaluate the modifications of half-sib family selection methodology to reduce selection cycle interval in *S. sphacelata* breeding programs.
2. To produce advanced populations of *S. sphacelata* selected for morphological and physiological traits of agronomic value.
3. To evaluate the effects of selection for morphological and physiological traits on agronomic performance in a common environment.

CHAPTER 3
DEVELOPMENT OF IMPROVED POPULATIONS IN *S. sphacelata*

Introduction

Breeding of any crop is a long process that involves many cycles of selection, controlled intermating, a good evaluation process and scrutinous eyes of the breeder. Many perennial warm-season forage species have the advantage of natural cross-pollination (Burton, 1982) thus eliminating the need of time consuming intermating by hand. Flowers and seeds in warm-season grasses are tiny and numerous which make emasculation an almost impossible task. Favored by self-incompatibility, random mating of inflorescences from selected parents has been successful when combined with multiple recurrent selection cycles to enhance the process of plant improvement.

In *S. sphacelata*, Hacker and Bray (1981) found high heritability for leaf width. Leaf width on vegetative tillers was positively correlated with flower head number and total dry matter yield. Flag leaf width was more correlated with number of days for flowering and flower head length, and negatively correlated with flower head number. There was no correlation with dry weight of plant. It appears that width of vegetative leaves is controlled by different gene systems than width of flag leaves. Selection for dry matter should, therefore, result in earlier flowering plants with shorter inflorescences and wider leaf blades (Hacker and Bray, 1981).

Hacker and Bray (1981) found that flower head number may easily be manipulated by the breeder. Flower head number was found to be highly heritable, and additive variance was strong and consistent. Hacker and Cuany (1997) obtained broad sense heritabilities for number of inflorescences plant^{-1} from 0.63 to 0.80 for four different cultivars. Van Wijk (1980) stated that in *Setaria*, selection for increased seed yield per plant would result in plants with higher number of flowering heads. Of the three components evaluated, head number, head length and 1000-grain weight, the first proved to be the most important as a determinant of seed yield.

Grazing results in changes in forage characteristics, as plants become adapted to the imposed management (Hodgkinson and Williams, 1983). Heavy grazing can increase the proportion of genotypes which are tolerant of that management (Charles, 1972). In *Setaria*, Hacker (1987) found that plants grown from commercial seed were more erect, variable in habit and taller than plants grown from experimental populations. He also found that a genetic shift to earlier flowering had occurred in populations subjected to heavy grazing pressures. However, genetic changes were small and probably would not affect animal or seed production.

The breeding program in Australia has resulted in successful transferance of genes for frost tolerance to superior agronomic cultivars (Hacker, 1972; Hacker et al., 1974; Hacker, 1985; Oram, 1990; Hacker, 1991a). Narok and Solander were bred for frost tolerance involving tetraploid accessions collected at 2200-m altitude in the Aberdares Region of Kenya. These high altitude accessions were shown to exhibit frost tolerance (Hacker et al., 1974).

Initial Population

A population of *S. sphacelata* was planted in August 1996 at the AFRU adjacent to the Dairy Research Unit of the University of Florida, near Gainesville, Florida. Two thousand seedlings were planted on 90-cm centers. The population was a bulk of seeds harvested by Dr. P. Mislevy at the Range Cattle Research and Education Center of the University of Florida, at Ona, from lines of *S. sphacelata* cvs. Narok, Kazungula and Solander and from roadside plants that had persisted along a drainage ditch for many years at the Research Center. This population served as the base population for all experiments. In September 1996, about 1000 seedlings of this same bulk of seeds were planted at Ona on 90-cm centers.

In the fall of 1996, grid selection for fall vigor was conducted in the base population at the AFRU. The field was divided into squares comprising 5 rows of five plants each. Two to three plants within each 25-plant square were selected for vigor, for a total of 200 plants, i.e., a selection pressure of 10% was imposed. Two inflorescences per selected plant were harvested once they were fully extended but before the flowers opened and any stigmas or stamens could be observed. If stigmas could be observed in any one flower in the inflorescence, then that inflorescence was not harvested. The inflorescences were cut from the base with roots and placed in a bucket of water in the greenhouse to be intercrossed in a polycross. They were covered with a large paper bag and shaken twice daily so that they would be randomly pollinated. When seeds were mature, each head was harvested individually and the seeds were threshed.

All lines were germinated, and further selection was made based on number of germinated seedlings (Figure 3.1). Germination was quite low overall. Twenty-four percent of the 200 lines did not germinate, and 29% germinated from 1 to 10 seedlings. A

total of eighty-eight percent of the lines germinated from 0 to 50 seedlings, while only 12% germinated from 50 to 100 seedlings. All the lines that had more than 30 seedlings were selected, for a total of fifty lines.

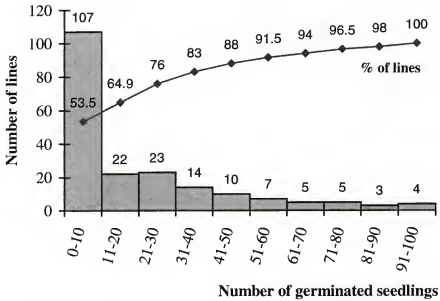

Figure 3.1 Number of germinated seedlings of 200 lines of *S. sphacelata* at Gainesville, Florida in 1997.

Twenty plants of each of the fifty selected lines were transplanted to the field on 22 Apr. 1997, in two replications of ten plants each. This population was designated VIGOR. The soil on which the experiment was grown was a Chipley sand (thermic coated Aquic Quartzipsamments). During June and July, these plants were evaluated for canopy plant height, reproductive plant height, flag leaf width, vegetative leaf width, inflorescence length and number of heads. The average canopy height was measured from the soil surface to the height where the majority of the leaves reached their maximum height. The reproductive height was measured from the soil surface to the top

of the inflorescences. Flag leaf width (the first leaf below the inflorescence) was measured in the middle of the leaf and three leaves per plant were used to obtain the mean. Vegetative leaf width was measured in the middle of fully extended leaves on vegetative culms. Three leaves per plant were used to obtain the mean. Inflorescence length was measured as a mean of five inflorescences and number of heads was estimated counting groups of 10 inflorescences.

Data were analyzed using General Linear Model procedure of SAS (SAS Institute, 1989), according to the model:

$x_{ijk} = \mu + R_i + f_j + rf_{ij} + e_{ijk}$

Where: x_{ijk} = effect of i_{th} replication and j_{th} family

μ = overall mean

R_i = fixed effect of i_{th} replication (i = 1-2)

f_j = random effect of j_{th} family (j = 1-50)

rf_{ij} = effect of interaction of i_{th} replication and j_{th} family

e_{ijk} = random effect of error

Heritability estimates were calculated on an individual plant basis and family selection according to Nguyen and Sleper (1983). The formulae used were:

1. Family selection $h^2 = \delta_F^2 / [\delta_F^2 + (\delta^2 / r) + \delta_w^2 / rn)]$
2. Individual plant basis $h^2 = 4\delta_F^2 / (\delta_F^2 + \delta^2 + \delta_w^2)$

where: δ_w^2 = variance among individual plants within plots

δ^2 = plot-to-plot environmental variance

δ_F^2 = family variance component

r=2 and n=10

Genetic gain was calculated according to the formula in Falconer and Mackay (1996):

$$\Delta G = h^2 (X_{pop} - X_{sel})$$

where: ΔG = genetic gain

h^2 = narrow sense heritability on an individual basis

X_{pop} = mean of the population

X_{sel} = mean of the selected population

The analyses of variance for each characteristic are presented in Table 3.1 and the estimate of the variance components and heritabilities in Table 3.2. Flag and vegetative leaf widths and inflorescence length showed the highest heritabilities, both calculated on a family selection and on an individual plant basis. Recurrent selection should thus result in greater improvement for these characteristics. Number of heads showed a heritability of 0.40 on an individual plant basis. However, this heritability could be an underestimate, since the evaluation was conducted at a very late date, when many plants were lodged and difficult to assess. The vegetative and reproductive plant heights showed the lowest heritabilities, suggesting that these traits vary not only due to their genotype, but also due to environmental effects, such as soil fertility and soil moisture.

Most characteristics evaluated showed broad phenotypic variability (Table 3.3), an indication that improvement through selection for these traits in this population is feasible. Vegetative plant height varied from 40 to 140 cm and inflorescence length varied from 7 to 38 cm. Number of heads per plant varied from 0 to 310, however, only five plants (0.5%) did not flower.

Table 3.1 Analysis of variance and expected mean squares for morphological characteristics in *S. sphacelata.*

	VH[+]		RH		FLW		
Source	**df**	**MS (P>F)**	**df**	**MS (P>F)**	**df**	**MS (P>F)**	**EMS**
Rep	1	0.0233 (0.30)	1	0.0720 (0.13)	1	0.3616 (0.01)	
Family	49	0.0876 (0.05)	49	0.0847 (0.04)	49	0.3857 (<0.01)	$\delta_w^2+n\delta^2+rn\delta_F^2$
Error (R x F)	49	0.0551 (<0.01)	49	0.0502 (<0.01)	48	0.0818 (0.01)	$\delta_w^2+n\delta^2$
Within (error)	838	0.0209	833	0.0307	828	0.0533	δ_w^2
Total	937		932		926		

(cont)

	VLW		INFL		HEAD		
Source	**df**	**MS (P>F)**	**df**	**MS (P>F)**	**df**	**MS (P>F)**	**EMS**
Rep	1	0.1793 (0.02)	1	540.434 (<0.01)	1	10479.825 (0.02)	
Family	49	0.1772 (<0.01)	49	91.322 (<0.01)	49	4867.899 (<0.01)	$\delta_w^2+n\delta^2+rn\delta_F^2$
Error (R x F)	49	0.0594 (<0.01)	47	22.538 (<0.01)	49	2702.762 (<0.01)	$\delta_w^2+n\delta^2$
Within (error)	820	0.0314	814	13.365	838	886.855	δ_w^2
Total	919		911		937		

[+]VH = vegetative plant height (m); RH = reproductive plant height (m); VLW = vegetative leaf width (cm); INFL = inflorescence length (cm); HEAD = number of heads.

Table 3.2 Estimate of variance components and heritability of morphological characteristics in *S. sphacelata*.

Morphological characteristic	Variance Component			Heritability *	
	δ_w^2	δ^2	δ_F^2	Indiv. pl.	Fam. sel.
Vegetative height	0.0209	0.0034	0.0016	0.25	0.37
Reproductive height	0.0307	0.0019	0.0017	0.20	0.41
Flag leaf width	0.0533	0.0029	0.0152	0.86	0.79
Vegetative leaf width	0.0314	0.0028	0.0059	0.59	0.67
Inflorescence length	13.365	0.917	3.439	0.78	0.75
Number of heads	886.85	181.59	108.26	0.37	0.44

* Heritability calculated on an individual plant basis and family selection basis

Highest correlation was found between vegetative and reproductive plant heights (r=0.53) (Table 3.4) and between vegetative and flag leaf widths (r=0.54). Inflorescence length had lower but significant correlation with all other traits.

Based on the preliminary evaluation, a decision was made to pursue recurrent selection breeding for increased head number (two cycles) and increased vegetative leaf width (one cycle). The methodology used was a modification of half-sib family selection with some restrictions suggested by Burton (1982) such as the use of detached culms and intermating under a large paper bag permitting control of male and female gamete sources in the production of the selected population. Half-sib progeny identification was maintained in the selected population and half-sib families were planted in the next generation. Half-sib family means were determined for the traits of interest and selection

in the next generation was based on superior half-sib families. In addition, further selection was conducted by selecting superior individuals within superior families. This methodology has been described as best plants in best families (Nguyen and Sleper, 1983).

Table 3.3 Mean and range of variability of morphological characteristics in *S. sphacelata.*

Morphological characteristic	Mean ± st. dev.	Range of variability
Vegetative height (cm)	109 ± 16	40 to 150
Reproductive height (cm)	176 ± 19	90 to 240
Flag leaf width (cm)	1.2 ± 0.3	0.5 to 2.2
Vegetative leaf width (cm)	1.0 ± 0.2	0.5 to 2.1
Inflorescence length (cm)	21.2 ± 4.3	7 to 38
Number of heads per plant	112 ± 35	0 to 310

Table 3.4 Correlation between morphological characteristics in *S. sphacelata.*

Morphological characteristic	VH	RH	FLW	VLW	INFL
Vegetative height (VH)					
Reproductive height (RH)	0.53 [+]				
Flag leaf width (FLW)	0.17	0.20			
Vegetative leaf width (VLW)	0.08 [#]	0.08 [#]	0.54		
Inflorescence length (INFL)	0.23	0.30	0.43	0.32	
Number of heads / plant (HEAD)	0.14	0.07 [$]	- 0.18	- 0.19	- 0.22

[+] all correlations have probabilities < 0.01 unless indicated
[#] Probability = 0.04
[$] Probability = 0.02

Heritabilities were calculated on an individual plant basis, and where these values were low, as a family selection. Predicted genetic gains were calculated based on the selection differential and heritability values. Both were calculated according to formulas described in this chapter.

Selection for Increased Head Number

The fifty half-sib families in the VIGOR population varied in their mean for 20 progenies, from 76 to 147 heads. Twenty families with the highest mean number of heads were selected. The selected families varied from mean 113 to 147 heads. Selection was carried out according to the methodology described earlier. In November 1997, three inflorescences from the five progenies with highest head numbers in each selected family were harvested for intercrossing in a polycross. Inflorescences were intercrossed in the greenhouse under a large paper bag as described earlier.

Threshed seeds of Cycle 1 were germinated in flats in the greenhouse in January 1998. On 26 March, 36 half-sib families that had more than 30 progenies were transplanted to the field on 90-cm centers in a randomized complete block design with two replications of 15 plants each. The soil type for this selection field and all selection fields hereafter was a Pomona sand (sandy, siliceous, hyperthermic Ultic Haplaquod). On 16 June, plants were evaluated for number of heads. The mean and standard deviation of the 36 half-sib families was 28 ± 20. The family mean number of heads plant^{-1} varied from 8 to 48. Number of heads was believed to be lower in this selection than the one in the fall of 1997 because the plants were much younger in this field (3 mo) than in the previous field (4.5 mo). Eighteen families were selected with a mean family number of

heads per plant of 28 to 48. As with the previous cycle, the five progenies with highest number of heads within each selected family were harvested and allowed to intercross in the greenhouse.

Threshed seeds of Cycle 2 were germinated in the greenhouse in February 1999. On 2 Apr. 1999, the 36 half-sib families that had more than 30 progenies were planted in the field on 90-cm centers in a randomized block design with 2 replications. On 16 June 1999, plants were evaluated for number of heads. The half-sib families varied from 40 to 133 heads, with a mean and standard deviation of 70 ± 28. Seventeen half-sib families were selected, varying from 70 to 124 heads per plant per family. Three inflorescences of the five plants with highest number of heads within each selected half-sib family were harvested and allowed to intercross in the greenhouse as described earlier. Seeds harvested from this polycross became Cycle 3 selected for increased number of heads. The analyses of variance for head number in both cycles are in Table 3.5 and the components of variance and heritability are in Table 3.6.

Contrary to suggestions in the literature (Falconer and Mackay, 1996), heritability estimates increased with each successive cycle of selection for number of heads. No decrease in variability was observed, probably due to the intermating technique used. The intercrossing of detached culms in close proximity under a large paper bag may have maximized gamete recombination and minimized inbreeding. Other researchers using similar intermating techniques have not seen a decrease in variability with this methodology (Burton, 1982).

Table 3.5 Analysis of variance and expected mean squares in *S. sphacelata* populations selected for increased number of heads.

	Head 1		Head 2		
Source	**df**	**MS (P>F)**	**df**	**MS (P>F)**	**EMS**
Rep	1	6550.90 (0.001)	1	7609.66 (0.001)	
Family	35	2582.20 (0.001)	34	5373.61 (0.001)	$\delta_w^2+n\delta^2+rn\delta_F^2$
Error (R x F)	35	1077.09 (0.001)	34	1289.83 (0.001)	$\delta_w^2+n\delta^2$
Within (error)	879	288.62	861	549.93	δ_w^2
Total	950		930		

Table 3.6 Estimate of variance components and heritability in *S. sphacelata* populations selected for increased number of heads.

	Variance component			Heritability ‡	
Cycle of breeding	δ_w^2 †	δ^2	δ_F^2	**Fam. sel.**	**Ind. pl.**
Head 1	288.62	52.57	50.28	0.58	0.51
Head 2	549.93	49.33	136.16	0.76	0.74

† δ_w^2 = variance among individual plants within plots; δ^2 = plot-to-plot environmental variance and δ_F^2 = family variance component.
‡ Heritability estimated as family selection and individual plant basis.

In Table 3.7, it can be observed that plants selected in each cycle always had more heads than the mean for that population, and with the exception of Cycle 3, the plants with the highest number of heads were selected. In Cycle 3, this was not possible, because the family that contained the plants with the highest head numbers, presented a lower family mean. Gains of 21, 66 and 45% can be predicted for these cycles of selection (Table 3.7).

Table 3.7 Means of populations and selections, heritability and expected gains for *S. sphacelata* populations selected for increased number of heads.

	Population		Selection			Expected gain	
Cycle	Mean ± st. dev.	Family range	Mean	Family range	h^2 † family	Mean	%
	----------Number of heads plant^{-1} ----------					no. heads pl^{-1}	%
0	112 ± 35	76 to 147	162	113 to 147	0.47	135	20.9
1	28 ± 20	8 to 48	60	28 to 48	0.58	46	65.5
2	70 ± 28	40 to 133	112	70 to 124	0.76	102	44.7

† Heritability estimated as family selection.

Selection for Increased Vegetative Leaf Width

The fifty half-sib families in the population VIGOR varied in vegetative leaf width from 0.8 cm to 1.3 cm and had a mean of 1.0 cm (Table 3.8). Nineteen families with the greatest leaf widths were selected to be intercrossed in a polycross. The selected families varied in width from 1 to 1.3 cm. The selected plants had a mean of 1.3 cm and varied from 1 to 2.1 cm. In June 1998, five inflorescences were harvested from each of five plants with the widest leaves within each selected half-sib family and allowed to intercross in the greenhouse, as described earlier. Seeds harvested from the polycross became cycle 1 selected for increased leaf width.

As mentioned earlier, vegetative width of leaves had a high heritability value of 0.69. Thus gain from one cycle of selection may be expected. With this population, the predicted gain was 16.2% (Table 3.8).

Table 3.8 Means and range of population and selection, heritability and expected gains for the *S. sphacelata* population selected for increased width of vegetative leaves.

	Population		Selection			Expected gain	
Cycle	Mean ± st. dev.	Family range	Mean	Family range	h^2 † family	Mean	%
	----------cm ----------					--cm--	%
0	1.0 ± 0.2	0.8 - 1.3	1.3	1.0 - 1.3	0.69	1.2	16.2

† Heritability estimated as family selection.

Selection for Grazing Tolerance

In September 1996, 1000 seedlings of the base population were planted on 90-cm centers at Ona, Florida. Plants with good forage characteristics were selected by mass selection, and open-pollinated seeds were harvested in January and June 1997. Selected plants were vigorous, with large crown area (more than 30 cm), presented a high percentage of regrown tillers, and an abundance of leaves, and were free of diseases on regrowth. Seeds of 95 selected plants were germinated in Gainesville, and ca. 1800 seedlings were sent back to Ona, where they were planted in late August 1997 on 40-cm centers in two replications. The area was mob grazed with yearling cattle to ca. a 10-cm height every 4 to 5 wks, with a total of five grazings from 7 Oct. 1997 to 30 Mar. 1998. After the regrowth and emergence of the inflorescences, vigorous plants were selected. Since the field was a sward, the identity of the half-sib families was not maintained, and selection was not made based on half-sib family means. Selection methodology was mass selection with no control of the male pollen. Selection was based on crown diameter, percentage of regrown tillers, height of regrowth and width of vegetative leaves. Five inflorescences of the best 150 plants were harvested in June and allowed to intercross in

the greenhouse, following the same procedure as described earlier. Seeds were germinated in Gainesville. Unfortunately, no seedlings emerged from the germinated seeds. It is speculated that flowers may have dried during the 3½-h drive from Ona to Gainesville and the subsequent very hot conditions in the greenhouse during the 1998 summer.

In this same summer of 1998, an attack of southern chinch bug [*Blissus leucopterus* (Homoptera: Lygaeideae)] killed almost all the plants of Setaria in the mob grazing trials and around 75% of the plants in the original nursery at Ona. The reasons for the greater development of chinch bug in one year in contrast to another are not known for certain, but hot dry weather has been seen to favor chinch bug development (Kuiter and Nutter, 1952). Chinch bug outbreaks usually occur in spring and early summer in Florida, especially when dry edaphic conditions exist. Therefore, severe setaria damage from this insect has been only observed when high chinch bug populations exist. The heavy and frequent rains that occur in summer help control the chinch bugs by killing the young insects and the eggs. In addition, the rainfall also favors the development and spreading of the fungi that kill the chinch bugs (Kuiter and Nutter, 1952). In 1994, a report was written documenting the increase in populations of chinch bug in the southeastern USA (Suszkiw, 1994).

The total annual rainfall in Ona in 1998 was very high (Kalmbacher, 1999). It was 371 mm higher than the 57-yr average and was only exceeded in 1962. Rainfall totals in February and March (Table A2) were record highs. However, from April to August, rainfall was below the 57-yr average. The spring season was particularly dry, with no rain having been recorded from 22 March to 20 April, and only 66 mm from 21 April to 31

May (Kalmbacher, 1999). These conditions appear to be ideal for the development of the chinch bug, and Setaria, as well as other grasses, was susceptible.

In September, seeds were harvested from the few surviving plants in the mob grazing trials, and in September and November, seeds were harvested from the surviving plants in the base nursery. Seeds of the surviving plants of the mob grazing trial and of the base nursery at Ona harvested in September were germinated and included in an experiment comparing populations (described in Chapter 4). These populations were named MOB and ONA, respectively.

Selection for Frost Tolerance

The base population for this selection was the source population (AFRU) planted at the AFRU in August 1996. The months of November through February had 1, 4, 5 and 1 d with minimum temperatures below 0°C, repectively. The minimum temperatures in December and January were -5 and -7°C, respectively.

On 4 Apr. and 2 May 1997, plants in the source nursery were evaluated for survival after the frosts. Approximately 25% of the plants in the field had died over the winter. The field was divided into grids of five rows of five plants each and the two most vigorous plants in each grid were selected. The vigor estimates were visually based and considered the general aspect of the plant, including its height, crown diameter, percentage of regrown tillers and abundance of leaves. At flowering in June, two inflorescences of each selected plant were harvested, removed and intercrossed in a greenhouse as described earlier. A total of 166 plants were harvested. Mature seeds of the two inflorescences per plant were combined and germinated. Only 38 lines produced 30 or more seedlings. This population was named FROST 1.

The 38 half-sib families in FROST 1 were transplanted to the field on 22 Sep. 1997 on 90-cm centers with 15 plants per family and two replications. Through December 1997, there were 4 d with minimum temperatures below 0°C and 16 d with minimums below 4°C. On 11 December, four days after a night with the minimum temperature of -4°C, visual evaluation for tissue damage after frosting was conducted. Plants were rated on a scale 1 to 5 (1= 100%, 2= 80%, 3= 60%, 4=40%, and 5= less than 20% of leaf tissue killed). Another 8 days with minimums below 0°C and 33 d with minimums below 4°C occurred from January to March 1998. On 22 Mar. 1998, plants were reevaluated for tissue damage. The 134 plants that had been rated 4 or 5 in the first evaluation and remained vigorous in the second were selected for intercrossing in a polycross. Three inflorescences of each selected plant were harvested for the intercross according to the procedure described earlier. The population resulting from this intercross was designated FROST 2.

Seeds of FROST 2 were germinated in the greenhouse. Lines that had germinated more than 60 seedlings were planted in two different fields. On 12 and 16 Oct. 1998, respectively, 38 half-sib families were planted at the AFRU and 32 half-sib families were planted at the North Florida Research and Education Center at Quincy, Florida. This latter field was named FROST Q0. In Gainesville, there were 10 d with minimum temperatures below 0°C and 17 d with minimum temperatures below 4°C through 12 Jan. 1999. On 25 and 26 January, minimum temperatures reached 3 to 4°C with no frost. After 13 Feb. 1999, there were further 9 and 22 d with minimum temperatures below 0 and 4°C, respectively (Table A1). In Quincy, there were 3 d with minimum temperatures below 0°C in December, 4 d in February and 1 d in March (Table A3).

Evaluation for spring survival and regrowth was conducted on 23 Jan. 1999 at the AFRU and on 27 Jan. 1999 at Quincy. Plants were evaluated following the scale 0 to 8 (0 = no regrowth to 8 = maximum regrowth). At the AFRU, family means varied from 3 to 5.4 and at Quincy from 3.4 to 6. On 17 May 1999, plants were selected at AFRU for survival after frost based on the visual classification and on physical appearance for vigor. Only plants with high visual scores were selected, but they also had to have high vigor. The best five plants of the best 20 families were selected. On 27 May 1999, the five best plants in the best 15 families were selected and harvested at Quincy. Three inflorescences of each selected plant were harvested at the AFRU and at Quincy and allowed to intercross in Gainesville and Quincy, respectively. Mature seeds were FROST 3 and FROST Q1, respectively.

The analyses of variance for FROST 1, FROST 2 and FROST Q0 are in Table 3.9 and the components of variance and heritability are in Table 3.10. Heritability estimates at the AFRU varied from 0.39 to 0.41 in the two cycles (Table 3.10). These heritability estimates are still high enough to suggest that there is possibility for further selection. Predicted gain in each cycle of selection was from 10 to 14% (Table 3.11).

Table 3.9 Analysis of variance for survival after frost in *S. sphacelata* populations.

	FROST 1		FROST 2		FROST Q0		
Source	**df**	**MS**	**df**	**MS**	**df**	**MS**	**EMS**
Rep	1	54.080 $^{+}$	1	17.733 $^{+}$	1	3.678 $^{\#}$	
Family	37	8.964 $^{+}$	37	9.464 $^{+}$	31	9.423 $^{+}$	$\delta_w^2+n\delta^2+rn\delta_F^2$
Error (RxF)	28	5.328 $^{+}$	37	5.796 $^{+}$	29	6.131 $^{+}$	$\delta_w^2+n\delta^2$
Within error	745	1.966	1044	1.870	771	1.597	δ_w^2
Total	812		1119		832		

$^{+}$ $P < 0.01$ and $^{\#}$ $P = 0.13$

Table 3.10 Estimate of variance components of survival after frost in *S. sphacelata* populations selected for frost tolerance.

	Variance component			Heritability ‡	
Cycle of breeding	δ_w^2 †	δ^2	δ_F^2	**Fam. sel.**	**Ind. pl.**
FROST 1	1.966	0.224	0.121	0.41	0.21
FROST 2	1.870	0.263	0.122	0.39	0.22
FROST Q0	1.597	0.302	0.110	0.35	0.22

† δ_w^2 = variance among individual plants within plots; δ^2 = plot-to-plot environmental variance and δ_F^2 = family variance component.
‡ Heritability estimated as family selection and individual plant basis

Table 3.11 Mean of population and selected plants, heritability and expected gains in *S. sphacelata* populations selected for frost tolerance.

Population	**Mean ± st. Dev**	**Selected mean**	**Visual scale †**	**h^2 ‡ family**	**Expected gain**	
	----------	**Grade**	----------		**Mean**	**%**
FROST 1	6.9 ± 1.6	8.6	1 to 10	0.41	7.6	10.1
FROST 2	4.5 ± 1.5	6.1	1 to 8	0.39	5.1	14.1
FROST Q0	5.1 ± 1.4	6.6	1 to 8	0.35	5.6	10.4

† Scale 1 = dead plant to 8 or 10 maximum = green plant
‡ Heritability estimated as family selection

In Quincy, heritability in cycle Q0 was 0.35 (Table 3.10), showing there is still possibility of selection for this trait. Predicted gain from one cycle of breeding in Quincy should be 10% (Table 3.11).

Summary and Conclusions

The evaluation of the initial setaria population at the AFRU suggested ample variability for reproductive and vegetative plant heights, flag and vegetative leaf widths, inflorescence length and number of heads. This also suggested that selection within this population should be feasible for any of these characteristics. However, selection would

probably be most effective for the traits flag and vegetative leaf width, inflorescence length and number of heads since these traits showed higher heritabilities. Variability for frost survival was also observed after the first winter of 1996-1997. Correlation of reproductive plant height with average canopy height and between vegetative and flag leaf widths was high, indicating that selection for one of these traits would affect the other trait. Inflorescence length had a low but significant correlation with all other traits.

The use of the modification of half-sib family selection methodology with some restrictions suggested by Burton (1982), permitted intercrossing of selected plants in the greenhouse, thus eliminating the need to plant isolation blocks of the selected plants in the field. With this methodology, improved populations were developed relatively easily and inexpensively. Despite the pollen contribution from all inflorescences for fertilization, caution must still be taken with this species to avoid inbreeding over time because of the elimination of some families due to poor germination. The elimination of families that do not germinate will increase the difficulty of calculating an inbreeding coefficient because some females are not included, leading to more inbreeding. In the present research, a total of eight populations were developed in a 3-yr period (three for increased head number, one for increased leaf width, one for grazing tolerance and three for frost tolerance). It was possible to obtain one cycle per year from recurrent selection. For the selections that were made in summer, it was possible to plant the population in spring, select in summer, intermate in the fall and germinate the seeds in the green-house in winter. For the frost tolerant populations it was possible to plant the populations in the fall, evaluate in the winter and spring, intermate in the spring, germinate those seed in the green-house in summer and reestablish a selection nursery in the fall.

The predicted gains obtained with each cycle of selection for each characteristic are optimistic. However, to prove that these gains are possible, the improved populations must be compared to the original populations in a common experiment in one environment with the same methodology. Remnant seed of these populations were used to establish such experiments and the results are reported in Chapters 4 and 5.

CHAPTER 4
COMPARISON OF *S. sphacelata* POPULATIONS SELECTED FOR MORPHOLOGICAL AND AGRONOMIC CHARACTERISTICS

Introduction

Cow-calf production is a very important enterprise in the southern USA and depends almost entirely on pastures. However, in Florida, pasture production is limited by the climate, which is too cold in the winter for growth of warm-season grasses and too hot in the summer for growth and persistence of cool-season grasses. The warm-season grasses that exhibit some degree of frost tolerance, such as limpograss (*H. altissima*), present problems of low forage N concentration (Sollenberger et al., 1988). *S. sphacelata* is a warm-season grass that has potential to increase the efficiency of beef cow-calf systems in southern USA, especially in Florida. It exhibits some degree of frost tolerance, is adapted to waterlogged conditions, and is productive and persistent (Hacker and Jones, 1969).

In order to study the performance of *S. sphacelata* under Florida conditions, and the possibilities of improvement through breeding, three populations were developed at the AFRU in Gainesville, and one population at the Range Cattle REC in Ona, Florida. These populations are two cycles of selection for increased number of heads, one cycle for increased width of leaves and one population selected under grazing. Predicted gains for the populations were promising, but to assess if the prediction can be translated into

realized gains, the populations must be compared to the initial populations simultaneously in the same environment.

The objectives of this study were to evaluate the four bred populations for various morphological and agronomic traits in comparison to the initial populations and standard commercial cultivars. Evaluation was conducted in a spaced-plant experiment and in a closely-spaced sward.

Material and Methods

The breeding populations selected for increased head number, Cycle 1 (HEAD1) and 2 (HEAD2), Cycle 1 selected for increased leaf width (LEAF) and the mob grazing population selected under grazing at Ona (MOB) were compared in two experiments. The standards for the comparison were the base population at the AFRU (AFRU), the vigor selection population described in Chapter 3 (VIGOR), the base population at Ona (ONA), the cultivars Solander and Splenda and the plant introductions CPI 33452, Fenwicks and CPI 33453, Thompson Falls. The latter four were received from the Australian Tropical Forages Genetic Resource Centre (ATFGRC). The CPI 33452 is a tetraploid from Kenya, and has shown an intermediate degree of frost tolerance in Australia. The CPI 33453 is a hexaploid from the high altitude Aberdares region in Kenya, and was tolerant to frost in Australia (Hacker, 1972)

Two experiments were planted at the FRU on 26 July 1999 with seedlings grown from remnant seed of the above populations. The soil type in the experiment was Pomona sand (sandy, siliceous, hyperthermic Ultic Haplaquod). The fields were fertilized with 25 kg N ha^{-1} at planting and irrigated. In the first experiment, 64 seedlings of the populations HEAD1, HEAD2, LEAF, MOB, ONA, AFRU, VIGOR and Solander were transplanted

to the field in a randomized block design with four replications. The plots consisted of four rows of four plants each spaced 90-cm between rows and plants. In September, the plants were evaluated for the morphological characteristics vegetative plant height, reproductive height, vegetative leaf width, inflorescence length and number of heads according to the methodology described in Chapter 3. When seeds were mature, seed production was evaluated. Three inflorescences of each of 16 plants in a replication were harvested and combined, threshed and analyzed for purity by running seeds in an air column seed blower to separate filled caryopses from empty caryopses and other detritus. One gram of each population within each replication was germinated in flats with potting mix in the greenhouse and the numbers of emerged seedlings were counted. Data were analyzed with Proc GLM of SAS (SAS Institute, 1989) according to the model described in Chapter 3.

In the second experiment, seedlings of all the populations above with the exception of MOB were transplanted to the field in a randomized complete block design with three replications. Plots were five rows of 12 plants each spaced 30 cm between rows and plants. Plots were harvested on 2 Sept. and 13 Oct. 1999 and fertilized with 25 kg N ha^{-1} applied as ammonium nitrate after each cut. Harvest consisted of mowing the end border row of each plot to a 10-cm height and then removing the material. The remaining three middle rows of 10 plants each (0.9m X 3.0m), were mowed to a 10-cm height, weighed in the field, a subsample of approximately 600 g was taken and weighed fresh in the field. The subsamples were then separated into leaves and stems. Leaf sheath was included with stems. Subsamples were forced-air dried at 60^0C for 72 h, weighed and ground in a Wiley Mill, to pass through a 1-mm screen. Leaf percentages were calculated

by dividing the dried leaf fraction by the sum of the dried leaf and stem fractions and multiplying by 100. The same procedure was used for calculation of stem percentages.

Ground leaves and stems were analyzed for CP concentration and in vitro organic matter digestibility (IVOMD) in the Forage Evaluation Support Laboratory of the Agronomy Department. In vitro organic matter digestion was performed by a modification of the two-stage technique (Moore and Mott, 1974). For N analysis, samples were digested using a modification of the aluminum block digestion procedure of Gallaher et al. (1975). Sample weight was 0.25 g, catalyst used was 1.5 g of 9:1 K_2SO_4:$CuSO_4$, and digestion was conducted for at least 4 h at 375°C using 6 mL of H_2SO_4 and 2 ml H_2O_2. Nitrogen in the digestate was determined by semiautomated colorimetry (Hambleton, 1977). Whole-plant IVOMD and CP were calculated from the laboratory data for leaf and stem and leaf and stem percentages in the dry matter harvested.

Total, leaf and stem dry matter yields, leaf percentage and leaf:stem ratio, leaf, stem and whole-plant CP concentration and IVOMD were analyzed as a split-plot in time design with Proc GLM of SAS (SAS Institute, 1989). The model used was:

$$x_{ijk} = \mu + R_i + p_j + rp_{ij} + h_k + ph_{jk} + e_{ijkl}$$

Where: x_{ijkl} = effect of i_{th} replication, j_{th} family and k_{th} harvest

μ = overall mean

R_i = effect of i_{th} replication (i = 1-3)

p_j = effect of j_{th} population (j = 1-10)

rp_{ij} = effect of interaction of i_{th} replication and j_{th} population

h_k = effect of k_{th} harvest date

ph_{jk} = effect of interaction of j_{th} population and k_{th} harvest date

e_{ijkl} = random effect of error

Population was tested with replication x population as the error term. Means were compared using Waller-Duncan multiple comparison of means.

Results and Discussion

In the spaced planting experiment, broad variability was found for each morphological characteristic evaluated (Table 4.1). The tallest plants were 2-m tall and maximum growth of leaves occurred up to 1.3 m. Leaf width varied from 0.57 to 2.13 cm and inflorescence length from 11 to 36.7 cm. Six plants (1.3%) did not flower and the maximum number of seed heads per plant was 145.

Table 4.1 Means and variation in morphological characteristics in seven selected populations and one cultivar of *S. sphacelata*.

Characteristic	Mean ± st. dev.	Range
VH[+] (cm)	87.9 ± 14.5	40 – 130
RH (cm)	154.4 ± 17.9	90 – 200
VLW (cm)	1.22 ± 0.26	0.57 – 2.13
INFL (cm)	20.3 ± 4.0	11 – 36.7
HEAD (no)	56.3 ± 27.8	0 – 145

[+]VH = vegetative plant height; RH = reproductive plant height; VLW = vegetative leaf width; INFL = inflorescence length; HEAD = number of heads.

The correlation between vegetative and reproductive heights was 0.59 ($P < 0.01$) but low correlation was found between the other variables (Table 4.2). Correlation values agree with those obtained for the initial population and reported in Chapter 3. The

correlation of leaf width with inflorescence length was 0.32 in both experiments. The correlation of number of heads with leaf width and inflorescence length was low and negative (-0.14). In the initial population, these values were -0.19 and -0.22, respectively. These values also agree with those of Hacker and Cuany (1997), who obtained a mean correlation of -0.17 between head length and head number for four cultivars of setaria in Australia, despite a large variation for this correlation between harvests. In a breeding experiment in Kenya, this correlation was very low (0.028) (van Wijk, 1980).

Table 4.2 Correlation between morphological characteristics in seven selected populations and one cultivar of *S. sphacelata*.

Morphological characteristic	**VH**	**RH**	**VLW**	**INFL**
Vegetative height (VH)				
Reproductive height (RH)	0.59 [+]			
Vegetative leaf width (VLW)	0.16	0.16		
Inflorescence length (INFL)	0.18	0.21	0.32	
Number of heads / plant (HEAD)	0.08 [#]	0.25	- 0.14	- 0.14

[+] all correlations have probabilities < 0.01 unless indicated
[#] P = 0.014

The analysis of variances for the morphological characteristics are presented in Table 4.3. Differences among populations occurred for all the characteristics evaluated.

Table 4.3 Mean squares and (P > F) from the analysis of variance for morphological characteristics in seven selected populations and one cultivar of *S. sphacelata*.

Source	df	VH[+]	RH	VLW	INFL	HEAD
Rep	3	0.006 (0.83)	0.057 (0.13)	0.223 (<0.01)	37.398 (0.05)	6437.157 (<0.01)
Pop	7	0.071 (<0.01)	0.148 (<0.01)	1.100 (<0.01)	115.986 (<0.01)	1882.369 (0.01)
Error	462	0.020	0.030	0.053	14.311	720.491

[+]VH = vegetative plant height, m; RH = reproductive plant height, m; VLW = vegetative leaf width, cm; INFL = inflorescence length, cm; HEAD = number of heads.

Comparisons of population means for the morphological characteristics showed that the tallest populations were AFRU, ONA, VIGOR and LEAF (Table 4.4). Solander had a high vegetative growth but not reproductive. Except for LEAF, there was a tendency for the selected populations (HEAD1, HEAD2 and MOB) to be shorter than the initial populations. The shortest populations were HEAD2 and MOB. This was expected for the grazed population and agrees with Hacker (1987) who found that *Setaria* plants grown from commercial seed were more erect and taller than grazed populations.

Table 4.4 Means of morphological characteristics in seven selected populations and one cultivar of *S. sphacelata*.

Population	VH[+]	RH	VLW	INFL	HEAD
	---------- cm ----------				-- no. --
AFRU	88 abc[#]	161 a	1.20 b	21.0 ab	55.6 a
ONA	92 a	157 abc	1.08 c	20.1 b	59.0 a
VIGOR	91 ab	159 ab	1.20 b	21.4 ab	54.1 ab
HEAD 1	86 bcd	154 bc	1.20 b	20.7 ab	57.3 a
HEAD 2	82 d	144 d	1.13 bc	17.5 c	63.9 a
LEAF	88 abc	155 abc	1.43 a	21.4 ab	57.2 a
MOB	83 cd	152 c	1.06 c	18.6 c	59.9 a
Solander	91 ab	152 c	1.41 a	21.6 a	43.5 b
Min Sign Diff	5	6	0.08	1.3	10.8

[+]VH = vegetative plant height; RH = reproductive plant height; VLW = vegetative leaf width; INFL = inflorescence length; HEAD = number of heads.
[#]Means followed by the same letter within a column do not differ significantly at P > 0.05, according to Waller-Duncan multiple mean comparison procedure.

Selection for increased leaf width was successful (Table 4.4). The LEAF population and Solander had significantly wider leaves than all other populations. The trait leaf width had a higher heritability than number of heads (0.67 vs 0.47, Table 3.2),

thus it was expected that one cycle of selection would significantly increase leaf width. In Australia, Hacker and Bray (1981) found high heritability estimates for leaf width and a positive correlation with flower head number. In the present experiment, this correlation was low and negative (- 0.14, $p<0.01$). The narrowest leaves were found in populations ONA and MOB. Selection for this trait increased the leaf width from 1.2 to 1.43 cm without changing the other measured morphological characteristics.

The longest inflorescences were found for Solander, however they only differed statistically from the populations HEAD2, MOB and ONA (Table 4.4). Populations HEAD2 and MOB presented the shortest inflorescences, therefore, selection for number of heads in the second cycle and for grazing tolerance resulted in a reduction in the length of the inflorescences. Inflorescence length in 121 setaria plants in Kenya varied from 7 to 25 cm with a mean of 17 cm. In Australia, the variation for four cultivars was from 18 to 22 cm, the same as obtained in the present experiment (Table 4.4). Solander exhibited a mean of 22 cm for its inflorescence length in Gainesville and Australia. This could indicate that this character is stable across environments. However, different values were reported (18 cm) (Hacker, 1991a), as well as an effect of N. At Samford, southeast Queensland, the application of 25 and 100 kg N ha^{-1} increased the length of inflorescence of Solander from 16 to 20 cm, respectively (Hacker, 1994).

Head numbers per plant varied from 44 to 64 for the different populations, a narrower range than the 29 to 71 heads per plant obtained by Hacker and Cuany (1997) for four cultivars. One of the cultivars, Solander, had 47 heads per plant, similar to the value (44 heads per plant) obtained in the present experiment. These were, however, the lowest values compared to the other populations. In Kenya, head number for 121 plants

varied from 42 to 377 (mean of 211 heads) and from 37 to 241 (mean of 129 heads) in two experiments (van Wijk, 1980). The level of N application largely influences number of seed heads (Hacker and Jones, 1971; Junqueira et al., 1985a; Cruz et al., 1989; Hacker, 1994; Dwivedi et al., 1999) and the effect of N on seed yield and seed head number was seen to be linear (Hacker and Jones, 1971). The level of N application may be the reason for such a high number of heads in the experiments in Kenya. Solander inflorescence numbers of 24 and 135 were reported with 25 and 100 kg ha^{-1}, respectively (Hacker, 1986). The level of N application and the soil-N level may have been responsible for the higher number of heads observed in the initial VIGOR population reported in Chapter 3 (variation of 0 to 310 heads, and mean of 112), as compared to the VIGOR population in the present experiment. Plants in the original selection field were more robust than in any of the subsequent populations.

The populations that were selected for number of inflorescences showed no differences for that trait (values of 57 and 64 in the successive cycles) when compared to the population from which they were selected (value of 54 for VIGOR) (Table 4.4). One possible reason for this is that the heritability estimates of 0.47 and 0.58 for Cycles 0 and 1, respectively (Table 3.7) were lower than of Hacker and Cuany (1997). They obtained estimates of 0.63 to 0.80 for four cultivars. However, their higher heritability estimates could also be due to use of broad-sense heritability values that are generally higher than narrow-sense heritabilities as calculated in this experiment. Selection for head number decreased plant height and inflorescence length in each cycle. It also decreased vegetative leaf width in the second cycle.

Selection for grazing tolerance (population MOB compared to ONA), resulted in a reduction in plant height and inflorescence length (Table 4.4). Vegetative leaf width and number of heads were not affected by selection for grazing tolerance.

Pure seed yield per head was higher for ONA (15.6 g 100 heads^{-1}), but it only differed from MOB and Solander (8.5 and 9.5 g 100 heads^{-1}, respectively) (Table 4.5). Seed weight per head was higher than reported by Hacker and Jones (1971). For two introductions, they obtained seed weight of 45 to 65 mg per head, while in our experiment the variation was from 85 to 156 mg per head.

Considering the number of heads produced by each plant (Table 4.4), the estimated production per plant varied from 4 (Solander) to 9.3 (ONA) g plant^{-1} or from 40 to 93 kg ha^{-1} estimated at 1 plant m^{-2} (Table 4.5). These values are in accordance with published levels of yield. In Australia, 11 populations varied from 51 to 293 kg ha^{-1} (Hacker, 1991b) and two introductions varied from 6 to 39 kg ha^{-1} (Hacker and Jones, 1971). In India, one cultivar yielded 70 kg ha^{-1} (Singh et al., 1995).

Pure seed yields of ONA and LEAF were significantly higher than yields of MOB and Solander (Table 4.5). The release of Solander was mainly based on consistently higher seed production when compared to Narok (Hacker, 1985 and 1994). Solander clean seed yields were reported as 8.7 and 57.7 kg ha^{-1} with 25 and 100 kg N ha^{-1}, respectively (Hacker, 1986), although there are reports of 100 kg ha^{-1} in the mean of two years (Hacker, 1991b). In the present experiment, Solander was estimated to have produced 40 kg ha^{-1} pure seed yields, however it was one of the lowest seed producers (Table 4.5). Seedling emergence was also higher for ONA, but did not differ from HEAD1 and MOB (Table 4.5).

Table 4.5 Pure seed yields and seedling emergence in seven selected populations and one cultivar of *S. sphacelata*.

Population	Pure seed yields			Seedling
	g 100 heads^{-1}	g plant^{-1} +	kg ha^{-1} +	emergence g^{-1}
AFRU	14.5 abc #	7.6 ab	76 ab	380 bc
VIGOR	14.1 abc	7.3 ab	73 ab	257 d
HEAD1	12.2 abc	6.9 abc	69 abc	394 abc
HEAD2	10.3 abc	6.5 abc	65 abc	372 bc
LEAF	15.5 ab	8.6 a	86 a	361 bc
ONA	15.6 a	9.3 a	92 a	461 a
MOB	8.5 c	5.0 bc	51 bc	427 ab
Solander	9.5 bc	4.0 c	40 c	323 cd
Mean	12.5	6.9	69	372

+ Estimate based on number of heads (Table 4.4) and production per head.
# Means followed by the same lower case letter within each column do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

For the sward-type experiment, results of the analysis of variance for total, leaf and stem dry matter yields, leaf percentage and leaf:stem ratio showed differences among populations for all variables but not among replications and no interaction occurred between replication and population (Table 4.6). Differences between harvests and its interaction with population occurred for all variables with the exception of leaf dry matter yield. Thus, the populations ranked differently in each harvest with the exception of leaf dry matter yield. The variables that showed interaction between populations and harvest (total and stem dry matter, leaf percentage and leaf:stem ratio) were therefore analyzed for each harvest. Differences among populations were found for all variables and for both harvests ($P<0.006$).

Total dry matter yields for the 10 populations varied from 2730 to 6550 kg ha^{-1} (Table 4.7). Levels of production are comparable to those obtained in Australia under grazing, where levels of 3510 to 4790 kg ha^{-1} (Jones and Evans, 1989) and of 3400 to 5280 kg ha^{-1} (Hacker and Evans, 1992) were obtained for three and four cultivars of setaria, respectively.

Population VIGOR followed by Splenda, AFRU and Solander were the most productive in the first harvest. These populations plus HEAD1 and ONA were the most productive in the total evaluation period. In the second harvest, however, populations ranked differently. ONA was the most productive, but only significantly higher than Thompson Falls and Fenwicks (Table 4.7). These latter two were always less productive than the other populations. In the first harvest, Fenwicks had a higher production than Thompson, but the reverse occurred in the second harvest. In the total harvests their production was similar and significantly lower than of the other populations.

The total dry matter production of the base populations AFRU and VIGOR, and the standards Splenda and Solander were much higher in the first harvest than in the second one, while production of the bred populations and of Ona were similar in both harvests (Table 4.7). Fenwicks produced twice as much in the first harvest as in the second one, while Thompson produced twice as much in the second harvest as in the first one. The first harvest was a growth period of 38 d after seedling transplant and the second harvest was a period of 41 d regrowth. Selection for number of heads decreased total dry matter production in the first harvest and the total of harvests.

Table 4.6 Mean squares and (P > F) from the analysis of variance for total, leaf and stem dry matter yields (Mg ha^{-1}), leaf percentage and leaf:stem ratio (L/S) in six selected populations and four cultivars of *S. sphacelata*.

Source	df	Total DM	Leaf DM	Stem DM	Leaf %	L/S
Rep	2	0.666 (0.12)	0.097 (0.22)	0.256 (0.18)	21.681 (0.49)	0.011 (0.88)
Pop [+]	9	2.033 (<0.01)	0.986 (<0.01)	0.820 (<0.01)	965.341 (<0.01)	1.193 (<0.01)
Error A (Rep*Pop)	18	0.380 (0.25)	0.123 (<0.06)	0.148 (0.43)	40.583 (0.24)	0.118 (0.25)
Harvest	1	2.947 (<0.01)	0.002 (0.67)	2.812 (<0.01)	400.290 (<0.01)	0.866 (<0.01)
Pop*Harvest	9	1.286 (<0.01)	0.091 (0.20)	0.743 (<0.01)	135.066 (<0.01)	0.336 (<0.01)
Error B	20	0.280	0.059	0.137	29.255	0.087
CV (%)		21.1	19.5	29.2	10.8	25.7

[+] P values derived from F = MS Pop / MS Error A

Table 4.7 Individual harvest and seasonal total dry matter yield of six selected populations and four cultivars of *S. sphacelata.*

Population	2 Sept.	13 Oct.	Total
	---------- kg ha^{-1} ----------		
AFRU	3440 abc ‡	2160 ab	5600 ab
VIGOR	4260 a	2290 ab	6550 a
HEAD1	2300 c	2790 a	5080 ab
HEAD2	2420 bc	2430 ab	4840 bc
LEAF	2590 bc	2420 ab	5010 b
ONA	2460 bc	2950 a	5410 ab
Solander	3160 abc	2300 ab	5460 ab
Splenda	3510 ab	2650 ab	6160 ab
Fenwicks	2290 c	1110 c	3400 cd
Thompson	920 d	1810 bc	2730 d
Mean	2730 A	2290 B	5020

‡ Means followed by the same lower case letter within a column or upper case letter within a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Seasonal leaf production varied from 690 to 3180 kg ha^{-1} (Table 4.8), a wider range than that range of 1340 to 1760 kg ha^{-1} obtained in Australia (Hacker and Evans, 1992). The population VIGOR had the greatest leaf dry matter production in the first and total growing period, however, it was only significantly different from HEAD1, Thompson and Fenwicks (Table 4.8). ONA had the greatest leaf production in the second harvest and it was only significantly different from Splenda, Thompson and Fenwicks. As with total dry matter production, Fenwicks produced twice as much leaf in the first harvest as in the second, while Thompson produced twice as much leaf in the second harvest as in the first. These two introductions had significantly lower leaf dry matter

yield at both harvests. The relationship of seasonal total and leaf dry matter yields can be seen in Figure 4.1. The contrast between the total dry matter production of VIGOR and Splenda as opposed to Fenwicks and Thompson is obvious. Also, leaf production in relation to total production in populations LEAF, HEAD2, ONA and Solander is in large contrast to this relation in populations Splenda, AFRU, HEAD1 and Fenwicks.

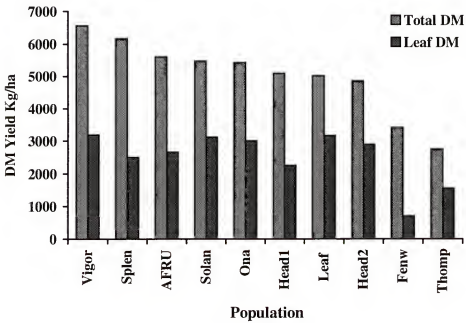

Figure 4.1 Total and leaf dry matter yields of six selected populations and four cultivars of *S. sphacelata.*

Stem production was greatest in Splenda (Table 4.8), but values were similar to the populations AFRU, VIGOR and HEAD1. Stem production varied between harvests and different populations ranked differently between each harvest. Stem production of Splenda was not different from that of AFRU, VIGOR, Solander or Fenwicks in the first harvest and HEAD1 and ONA in the second harvest.

Comparing harvests, values of leaf production obtained in the first harvest were similar to those obtained in the second harvest (Table 4.8). However, stem values for half of the populations in the first harvest were almost two to three-fold higher than in the second harvest. The decrease in the growth of total dry matter in some populations was, therefore, due to a decrease in stem regrowth rather than leaf regrowth. This suggests that under grazing, these populations could probably acquire a more prostrate habit than the others could.

Table 4.8 Individual harvest and seasonal leaf and stem dry matter yield of six selected populations and four cultivars of *S. sphacelata*.

	Leaf dry matter yield			Stem dry matter yield		
Population	**2 Sept.**	**13 Oct.**	**Total**	**2 Sept.**	**13 Oct.**	**Total**
	----------kg ha^{-1}----------					
AFRU	1350 ab [+]	1290 abc	2640 ab	2090 abc	870 c	2960 abc
VIGOR	1760 a	1420 abc	3180 a	2500 a	870 c	3370 ab
HEAD1	1030 bc	1210 abc	2240 bc	1270 cd	1580 a	2840 abcd
HEAD2	1450 ab	1430 abc	2880 ab	960 de	1000 bc	1970 def
LEAF	1550 ab	1600 ab	3150 a	1040 de	820 c	1860 fe
ONA	1360 ab	1640 a	3000 ab	1100 de	1320 ab	2420 cde
Solander	1650 a	1450 ab	3110 a	1510 bcd	840 c	2350 cde
Splenda	1320 ab	1170 bc	2490 ab	2190 ab	1480 a	3670 a
Fenwicks	480 c	210 d	690 d	1810 abcd	900 bc	2710 bcde
Thompson	560 c	980 c	1540 c	360 e	830 c	1190 f
Mean	1250 A	1240 A	2490	1480 X	1050 Y	2530

[+] Means followed by the same lower case letter within a column or upper case letter within a response variable in a row do not differ significantly at the $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Population LEAF had the highest leaf percentage (63%) and leaf:stem ratio (1.74), however, it was not different from HEAD2, Solander or Thompson (Table 4.9). All these populations had a leaf percentage higher than 58% and leaf:stem ratio higher than 1.42. The Populations HEAD1, Splenda and Fenwicks had a leaf:stem ratio less than 1.0. Fenwicks had a particularly low leaf:stem ratio, 0.25, which is equivalent to only 20% leaf. In a subtropical environment in Australia, cut every 6 wk and fertilized with superphosphate and KCl, stem percentages varied from 34 to 50% (Hacker, 1991a), which is equivalent to leaf percentages of 50 to 66%. In the present experiment, leaf percentages varied from 41 to 63% with the exception of Fenwicks with the same regrowth period. In Kenya, leaf percentage decreased from 60 to 40 to 24% with regrowth periods of 3, 6 and 9 wk, respectively, and in another experiment, from 62 to 32% with regrowth of 2 and 6 wk, respectively (van Wijk, 1980). However, higher leaf:stem ratios than the ones reported here however, were observed in a grazing trial in subtropical coastal lowlands of southeast Queensland. Four cultivars of setaria varied from 0.94 to 2.08 leaf:stem ratio, with a mean of 1.56 under rotational grazing. Measurements were made every 5 wk before the animals entered the pasture and 2 wk after they left the pasture (Hacker and Evans, 1992).

Selection for leaf width, therefore, increased the percentage of leaves in relation to the stems. However, leaf dry matter production was not increased. The increase in leaf percentage was due to a decrease in stem production in the first harvest (Table 4.8). Total dry matter production was not affected.

Table 4.9 Individual harvest and seasonal leaf percentage, and seasonal leaf/stem ratio of six selected populations and four cultivars of *S. sphacelata*.

	Leaf percentage			L/S ratio
Population	**2 Sept.**	**13 Oct.**	**Mean**	**Mean**
AFRU	39 c [+]	59 abc	49 cd	1.08 bc
VIGOR	42 bc	63 ab	53 bc	1.25 b
HEAD1	45 bc	43 d	44 de	0.79 c
HEAD2	60 a	59 abc	60 ab	1.47 ab
LEAF	60 a	66 a	63 a	1.74 a
ONA	56 a	56 bc	56 abc	1.32 b
Solander	52 ab	64 ab	58 ab	1.42 ab
Splenda	38 c	44 d	41 e	0.71 c
Fenwicks	21 d	19 e	20 f	0.25 d
Thompson	61 a	54 c	58 ab	1.44 ab
Mean	47 A	53 B	50	1.14

[+] Means followed by the same lower case letter within a column or upper case letter within a response variable do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

In Table 4.10 are the results of the analysis of variance for CP concentration and IVOMD in leaves, stems and whole plant of the 10 populations of *S. sphacelata*. There were no differences among populations for CP concentration of leaves and stems, but whole-plant CP differed among populations. In contrast, there were differences between harvests for leaf and stem CP, but not for whole plant. Interaction of harvest and populations occurred for stem and whole-plant CP.

Differences among populations occurred for leaf and stem IVOMD and whole-plant IVOMD and CP (Table 4.10). The IVOMD and CP in leaves and stems and whole-plant IVOMD also varied between harvests. Population only interacted with harvest for stem IVOMD and CP and whole-plant CP. Since interaction of populations with harvests occurred, CP and IVOMD in stems were analyzed for each harvest date. Differences

among populations for whole plant, leaf or stem CP only occurred in the first harvest date ($P < 0.05$). Differences among populations for stem and whole-plant IVOMD occurred in both harvests dates ($P < 0.05$).

Table 4.10 Probabilities of calculated F > table F for crude protein (CP) and in vitro organic matter digestibility (IVOMD) in leaves, stems and whole plant (WP) of six selected populations and four cultivars of *S. sphacelata*.

	CP			IVOMD		
Source	**Leaf**	**Stem**	**WP**	**Leaf**	**Stem**	**WP**
Rep	0.0001	0.0001	0.0001	0.0001	0.9529	0.0010
Pop	0.2659	0.1752	0.0198	0.0011	0.0062	0.0001
Rep*Pop	0.1595	0.6459	0.5254	0.0975	0.1754	0.0758
Harvest	0.0001	0.0069	0.9193	0.0001	0.0001	0.0001
Pop*Harvest	0.2989	0.0320	0.0091	0.8793	0.0258	0.1664

Means for leaf and stem CP at each harvest date are presented in Table 4.11. Leaf CP during the experimental period varied from 129 to 182 g kg^{-1}. Values in the first harvest (145 to 182 g kg^{-1}) were always higher than in the second harvest (129 to 163). Fenwicks followed by Splenda and HEAD1 had the highest values in the mean of harvests, and VIGOR the lowest, but no differences were found among them. Populations did not differ in the first harvest. In the second harvest, Splenda (166 g kg^{-1}) was only superior to VIGOR (134 g kg^{-1}) and Thompson (129 g kg^{-1}).

Over harvests, stem CP varied from 72 to 123 g kg^{-1}. For the mean over harvests, Thompson had the highest CP concentration (110 g kg^{-1}) but only differed from VIGOR, which had the lowest CP (87 g kg^{-1}). In the first harvest, Thompson had the highest CP, and it did not differ from HEAD1, LEAF and ONA. In the second harvest, LEAF had the

highest concentration, and it only differed from HEAD2. Comparing both harvests, populations AFRU, VIGOR, LEAF, Solander, Splenda and Fenwicks had lower concentrations in the first harvest than those of the second harvest. These populations had higher total dry matter yield in the first harvest when compared to the second harvest. Therefore, protein concentration was lower, where more growth occurred.

Table 4.11 Individual harvest and seasonal crude protein in leaves and stems of six selected populations and four cultivars of *S. sphacelata*.

	Leaf			Stem		
Population	**2 Sept.**	**13 Oct.**	**Mean**	**2 Sept.**	**13 Oct.**	**Mean**
	----------	----------	---------- g kg^{-1} ----------	----------	----------	----------
AFRU	154 a ‡	152 ab	153 a	72 c	114 a	93 ab
VIGOR	145 a	134 b	139 a	73 bc	100 ab	87 b
HEAD1	173 a	153 ab	163 a	102 ab	96 ab	99 ab
HEAD2	166 a	142 ab	154 a	92 bc	88 b	90 ab
LEAF	165 a	152 ab	158 a	94 abc	117 a	105 ab
ONA	163 a	138 ab	150 a	100 abc	100 ab	100 ab
Solander	161 a	156 ab	159 a	89 bc	110 a	100 ab
Splenda	169 a	163 a	166 a	84 bc	104 ab	94 ab
Fenwicks	182 a	155 ab	168 a	82 bc	102 ab	92 ab
Thompson	175 a	129 b	152 a	123 a	97 ab	110 a
Mean	165 A	147 B	156	91 Y	103 X	97

‡ Means followed by the same lower case letter within each column or upper case letter within a response variable do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Leaf IVOMD varied over harvests from 372 to 649 g kg^{-1} (Table 4.12). Fenwicks was significantly higher in IVOMD at both harvests (mean 573 g kg^{-1}). Ona had the lowest IVOMD. Stem IVOMD across harvests varied from 439 to 606 g kg^{-1}. With the exception of Thompson, stem digestibilities were always higher than leaf digestibilities.

This was not expected, because digestibilities in leaves were found to be 20 to 30 g kg^{-1} higher than in stems (Hacker et al., 1974). However, it agrees with Hacker (1971) in the sense that the low quality of setaria in comparison to temperate grasses results from low leaf digestibility and not low digestibility of the stem.

Leaf and stem digestibilities in the first harvest were always higher than in the second harvest. Fenwicks once again had the highest IVOMD, while Ona had the lowest. The IVOMD reported from 6-wk regrowth are somewhat lower than those obtained in Australia, where leaf digestibility of 35 setaria genotypes at 4-wk regrowth varied from 581 to 781 g kg^{-1}, and in the stem from 596 to 736 g kg^{-1} (Hacker et al., 1974).

Table 4.12 Individual harvest and seasonal In vitro organic matter digestibility in leaves and stems of six selected populations and four cultivars of *S. sphacelata*.

	Leaf			Stem		
Population	**2 Sept.**	**13 Oct.**	**Mean**	**2 Sept.**	**13 Oct.**	**Mean**
	---------- g kg^{-1} ----------					
AFRU	563 bc ‡	401 bc	482 bcd	547 cd	489 bc	518 bc
VIGOR	558 bc	405 bc	481 bcd	548 cd	499 abc	524 bc
HEAD1	554 bcd	420 bc	487 bcd	570 bc	487 bc	528 bc
HEAD2	551 bcd	439 abc	495 bc	595 ab	459 bc	527 bc
LEAF	531 cd	407 bc	469 cd	580 abc	467 bc	524 bc
ONA	511 d	372 c	442 d	535 d	456 bc	495 c
Solander	543 bcd	399 bc	471 cd	573 abc	439 c	506 c
Splenda	547 bcd	419 bc	483 bcd	587 ab	512 ab	550 ab
Fenwicks	649 a	497 a	573 a	606 a	552 a	579 a
Thompson	586 b	452 b	519 b	564 bcd	445 c	504 c
Mean	559 A	421 B	490	571 X	481 Y	526

‡ Means followed by the same lower case letter within a column or upper case letter within a response variable do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Leaf and stem IVOMD and CP were combined proportionally to their leaf and stem percentages and whole-plant IVOMD and CP were calculated. The CP values in the whole plant varied from 103 to 154 g kg^{-1} (Table 4.13). The population LEAF showed the highest concentrations (138 g kg^{-1}) followed by Solander and Thompson. These values only differed from those of VIGOR and Fenwicks. These CP values were found to be high in comparison to other tropical forage grasses which are known to frequently exhibit less than 60 g kg^{-1}, a value that is below the maintenance requirement of many classes of animals (Minson and Milford, 1967). These values were also much higher than the 75 to 88 g kg^{-1} values reported by Jones and Evans (1989) for three cultivars of setaria under grazing. However, values of 146 g kg^{-1} were reported in Brazil (Almeida and Flaresso, 1991). Protein decreases rapidly with plant maturity and other studies at the same age of regrowth have reported values of 95 g kg^{-1} in India (Singh and Pradhan, 1995), and always above 95 g kg^{-1} and frequently above 125 g kg^{-1} in Australia (Bray and Hacker, 1981).

Crude protein values in leaf, stem and whole plant are shown in Figure 4.2. Fenwicks had the highest leaf CP, but the lowest whole-plant CP. The populations that exhibited larger leaf percentage had a smaller difference between whole plant and leaf CP. Conversely, the populations that exhibited larger stem percentage, had a larger difference between whole plant and leaf CP. Whole-plant IVOMD values varied from 470 to 580 g kg^{-1} (Fig 4.3). Fenwicks had the highest IVOMD (579 g kg^{-1}) and it differed from all other populations. Higher digestibility levels than the ones obtained here were reported. In Australia, values varied from 508 to 710 for herbage harvested at 4-wk intervals (Hacker, 1974a) and from 587 to 625 g kg^{-1} for 13 populations harvested at 6-

wk intervals (Hacker, 1991a). However, digestibility has been found to decrease with the maturation of the plant. In Kenya, this decrease was from 637 to 568 to 510 g kg^{-1} with 3, 6, and 9 wk regrowth, respectively (van Wijk, 1980). In another experiment in Kenya, values obtained were 572, 566, 542, and 506 g kg^{-1} at 2, 4, 6 and 8 wk of regrowth, respectively, dropping to 420 and 358 g kg^{-1} at 12 and 16 wk regrowth, respectively (van Wijk, 1980). In Australia, six introductions planted at three sites had a decrease in digestibility from 618 g kg^{-1} with 4 wk regrowth to 540 and 503 g kg^{-1} with 8 and 12 wk regrowth, respectively (Hacker and Minson, 1972). At one site however, the lowest values were 470 g kg^{-1} averaged over harvests, so in one specific harvest, values could be even lower.

The values of digestibility obtained in the present experiment are on the lower end of values reported for tropical grasses. The regrowth period of 6 wk between harvests permitted the plants to mature and to flower. Flowering was very intense, especially in the second harvest. In this harvest, digestibility values were lower than in the first harvest. It is known that flowering also affects digestibility, and in Australia, values for Kazungula dropped from 660 g kg^{-1} before flowering to 400 g kg^{-1} after intense flowering (Moss and Murray, 1992).

First harvest IVOMD was higher than in the second harvest. The bred populations had similar values as the population from which they were selected, therefore, selection for head number or leaf width did not affect IVOMD.

Leaf, stem and whole-plant IVOMD may be seen in Figure 4.3. For most populations, stem IVOMD was larger than leaf IVOMD. For Fenwicks and Thompson, however, leaf and stem digestibilities were similar.

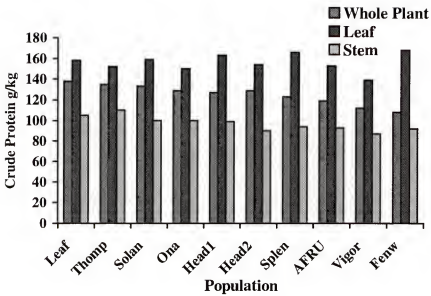

Figure 4.2 Crude protein concentration in the whole plant, leaves and stems of six populations and four cultivars of *S. sphacelata*.

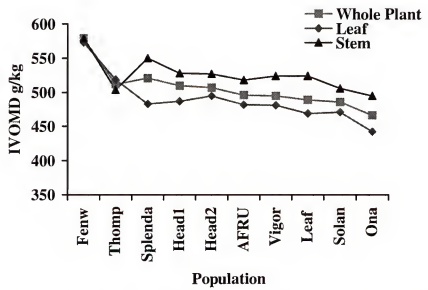

Figure 4.3 Whole-plant, leaf and stem in vitro organic matte digestibility (IVOMD) of six populations and four cultivars of *S. sphacelata*.

Table 4.13 Individual harvest and seasonal crude protein (CP) concentration and in vitro organic matter digestibility (IVOMD) in the whole plant of six selected populations and four cultivars of *S. sphacelata*.

	CP			IVOMD		
Population	**2 Sept.**	**13 Oct.**	**Mean**	**2 Sept.**	**13 Oct.**	**Mean**
	---------- g kg^{-1} ----------					
AFRU	104 c ‡	135 ab	119 abc	554 bc	439 bcde	496 bc
VIGOR	103 c	121 cd	112 bc	551 bc	439 bcde	495 bcd
HEAD1	134 ab	120 cd	127 ab	563 b	457 bc	510 bc
HEAD2	137 ab	120 cd	129 ab	568 b	446 bcd	507 bc
LEAF	136 ab	140 a	138 a	551 bc	427 cde	489 cd
ONA	136 ab	121 bcd	129 ab	524 c	407 e	466 d
Solander	127 abc	139 a	133 a	558 b	414 de	486 cd
Splenda	116 bc	130 abc	123 abc	572 b	470 b	521 b
Fenwicks	103 c	113 d	108 c	615 a	543 a	579 a
Thompson	154 a	115 d	135 a	574 b	449 bcd	512 bc
Mean	125	125	125	563 X	449 Y	506

‡ Means followed by the same small case letter within a column or upper case letter within a response variable in a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Summary and Conclusions

The objectives of this study were to compare improved populations of *S. sphacelata* in the same environment to quantify their performance and the gains obtained through selection relative to comercial cultivars and other populations. Selection for leaf width increased the width of leaves without changing the other measured morphological characteristics. Selection for leaf width also increased the percentage of leaves, leaf:stem ratio, and whole-plant CP concentration. However, stem and total dry matter yield were decreased, but leaf dry matter yield and IVOMD were not affected.

Increasing the width of leaves should increase sward density and therefore, short-term intake by animals. Intake was not measured in this experiment, but lines with a higher percentage of leaves and a decrease in stems observed in the leaf population may impact animal production. The decreases in stem and total dry matter production were not expected, but may have been due to more assimilates being partitioned from the stems to the leaves. In future studies, the effect of selection for increased leaf width on the structure of the plant should be determined to explain the reason for the decline in stem and total dry matter yields. Studies should investigate whether stem dry matter yield decreased because assimilates were partitioned to the leaves or whether other correlated changes occurred, such as a decline in number of tillers or in stem diameter. The reasons for leaf dry matter yield not increasing should also be considered. Possible reasons include a decrease in leaf length or in number of leaves when increasing the leaf width due to selection.

In *Hemarthria*, tetraploid plants had thicker stems than diploid plants. Contrary to the expected, tetraploid plants had higher IVOMD (Schank et al., 1973). The reason for the higher IVOMD was found to be the anatomical structure of the stems. Stems of tetraploid plants had less vascular bundles per area of stem than diploid plants. In this experiment, increasing leaf width could have reduced the number of vascular bundles per area of leaf and thus result in greater leaf CP and IVOMD concentration. However, a higher concentration of CP and IVOMD did not occur, and the increase in whole plant CP observed was because there were a higher percentage of leaves and these had higher CP concentration than the stems.

Further studies should be undertaken to understand why IVOMD values were in general low, and why values in the stems were higher than in the leaves. It is suggested that experiments be conducted to evaluate the effects of increasing applications of N, P and K on the nutritive value of setaria in the leaves, stems and in the whole plant. Fertilization rates could be 25 kg N ha^{-1} as used in this experiment, 50, 100 and 200 kg N ha^{-1}. Once an optimum maximum rate of N fertilization is assessed additional research to evaluate individual plants for IVOMD and determine the genetic variability for this trait within populations could be conducted. Other parameters such as crude protein, number of heads, leaf width, leaf and stem production, and number of vegetative and fertile tillers.

Selection for head number increased head number but decreased plant height, leaf width and inflorescence length in one or both cycles. The increase in number of heads did not result in an increase in seed production. Selection for number of heads decreased total and stem dry matter yields in the second cycle, and leaf dry matter yield, leaf percentage and leaf:stem ratio in the first cycle. The CP and IVOMD were not affected by this selection.

Selection for increased number of heads decreased height of the canopy, height of the inflorescence, inflorescence length and stem and total dry matter yields, probably because a partitioning occurred into increase number of heads. However, Hacker and Bray (1981) stated that selection for increased number of heads was an indirect method for increasing dry matter yield, due to an increase in weight of the stems. This data suggest that there was a compensating effect such that increasing head number resulted in a decrease in stem length.

Selection for regrowth after grazing resulted in reduced plant height and inflorescence length and reduced seed production. The effects on dry matter yield and nutritive value could not be determined because population MOB was not included in the sward experiment due lack of sufficient quantity of seeds.

The germplasm of *S. sphacelata* was plastic enough that modification in morphological and agronomic traits due to selection and breeding was successful mainly regarding improvement of width of leaves. Levels of dry matter production were comparable to other tropical pasture grasses, but CP concentration was higher. Levels of IVOMD, however, were lower than reported for Setaria grown in other locations. The conflict between nutritive results (high CP and low IVOMD) remain unresolved. The suggested research discussed in the previous paragraphs may help resolve these conflicts. Thus, although setaria appears to be a promising plant for Florida conditions, additional research to resolve these issues must be conducted. Concerns regarding susceptibility of *Setaria* to chinch bug and possible winter killing in Florida remain to be resolved before this species may be commercialized in the state of Florida. Additional evaluation of persistence under variable grazing management should be assessed before this grass may be recommended to farmers.

CHAPTER 5
COMPARISON OF FROST TOLERANT POPULATIONS OF *S. sphacelata*

Introduction

In the *S. sphacelata* complex, much variability for frost tolerance exists (Hacker, 1972; Davies and Forde, 1991). The cultivars Nandi and Kazungula are intolerant of frost, Narok is intermediate and introductions from the Aberdares region of Kenya are very tolerant (Hacker, 1972; Hacker et al., 1974). Frost resistance in *Setaria* has been associated with altitude of origin (Hacker et al., 1974). In 1963, a collection expedition focusing on genotypes tolerant of frost was conducted in Kenya by R.J. Jones (Hacker, 1966). In the mountainous areas in the Aberdares Range in Kenya, night temperatures fall below 0°C and introductions from this region were shown to exhibit frost tolerance (Hacker, 1972). Narok was the first wintergreen cultivar to be released (Oram, 1990). Its parental introduction was from the Aberdares Range and it was selected for winter yield and winter greenness in southeast Queensland, Australia (Hacker, 1972). The released cv. Solander was selected from a population derived from crosses involving two frost-resistant accessions (which contributed 16 and 34%, respectively, of the germplasm in the F1) with 3 robust, though frost-susceptible accessions (Oram, 1986).

In comparison to *P. clandestinum* in Australia, higher annual liveweight gains of Narok were associated with grass growth in low temperature and frost tolerance (Evans and Hacker, 1992b). The winter greenness of Solander was comparable to that of Narok

under conditions (-2 to -3.5°C) which killed 60 to 70% of leaves in Nandi and Kazungula (Oram, 1986). Despite Narok having been selected from a high-altitude introduction and being relatively frost resistant, it has a low chilling tolerance (Davies and Forde, 1991). High chilling tolerance, which is the tolerance to exposure to long periods of low temperatures after the frosts, has been shown to be important for the survival of frost-sensitive grasses.

In southern Brazil, the advantage of *S. sphacelata* was shown to be spring growth, while *H. altissima* was recommended for its autumn growth, in comparison to 34 other grasses (Rocha et al., 1994). In Japan, on the other hand, setaria showed a slow regrowth in early spring after frosting, which was related to an active growth in autumn. Many of the other tropical grass species that presented high spring regrowths showed depressed growth in autumn (Ito et al., 1985).

Three populations of *S. sphacelata* were developed at the AFRU near Gainesville, FL and one at the NFREC at Quincy, FL, with objectives of increasing the tolerance to frost and adaptation of the species to the cool winters of Florida. The development of the populations was described in Chapter 3. In the present study, these populations were compared to standard populations and cultivars in the same environment in two experiments, one in a spaced plant experiment comparing morphological traits and the other in a sward comparing agronomic traits.

Material and Methods

The frost tolerant populations developed at the AFRU (FROST 1, FROST 2 and FROST 3) and the population developed at Quincy (FROST Q1) (described in Chapter 3) were compared in two experiments. The standards for the comparison were the base

population at the AFRU (AFRU), the Australian cultivars Solander and Splenda and the plant introduction CPI 33453, Thompson Falls. The introduction Fenwicks was not included due to its very poor survival after mowing observed in the experiment in Chapter 4, despite it having demonstrated the most winter-greenness in Australia (Hacker personal communication, 1999).

On 6 Oct. 1999, seedlings grown from remnant seed of the above populations were transplanted to two different field experiments. The first was a close-spaced experiment (simulating sward) with plots of five rows of 12 plants each spaced 30 cm between rows and plants and replicated three times. The second was a wide-spaced experiment where 64 seedlings of each population were transplanted to the field in plots of four rows of four plants each spaced 90 cm between rows and plants and with four replications.

The cold days in the winter of 1999-2000 were more concentrated than the cold days in previous winters. The average minimum temperature in December was below the 70-year average, and in January and February average minimum temperatures were similar to the 70-year average (Figure 5.1). The winter 1999-2000 had 18 nights with temperatures below 0^0C and 38 nights with temperatures below 4^0C during the 84 days from 1 Dec. 1999 to 22 Feb. 2000. Evaluation for survival after this cold winter showed an overall survival rate of 11% in the close-spaced experiment and 32% in the wide-spaced experiment. Due to the overall poor survival rate, it was decided to replant this experiment in order to compare the populations. Remnant seeds of all populations were used, except Thompson Falls, which was not included due to its poor survival after the winter 1999-2000 in Gainesville (Table 5.13). The new experimental plots were

transplanted on 18 May 2000 with the same populations as the previous fields with the exception of Thompson Falls.

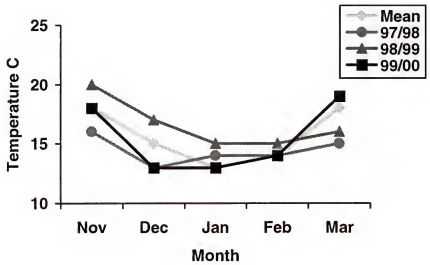

Figure 5.1 Average monthly minimum temperature in winter from 1997 to 2000 and 70-yr average in Gainesville, Florida.

In the wide-spaced experiment, plants were evaluated in September 2000 for the morphological characteristics vegetative plant height, reproductive height, vegetative leaf width, inflorescence length and number of heads, as described in Chapter 3. Data were analyzed using Procedure GLM of SAS (SAS Institute, 1989) according to the model described in Chapter 3.

In the close-spaced, sward-type experiment, plots were harvested for evaluation of forage dry matter yield and quality on 13 July and 30 Aug. 2000. The harvest procedure consisted of mowing the end border rows to a 10-cm height from each end of each plot and then removing the material. The remaining three middle rows of 10 plants each (0.9m

X 3.0m) were mowed to 10-cm height, weighed fresh in the field, a subsample of around 600 g was taken and weighed in the field. The subsamples were then separated into leaves and stems. Leaf sheath was included with stems. Due to the presence of weeds, weeds were also separated in the fresh subsample, weighed, dried and weighed for dry matter. Total, leaf and stem dry matter yields were reported in the non-weed fraction, as well as leaf percentages and leaf:stem ratios. Subsamples were forced-air dried at 60^0C for 72 h, weighed and ground in a Wiley Mill, to pass through a 1-mm screen.

Ground leaves and stems were analyzed for crude protein and IVOMD in the Forage Evaluation Support Laboratory of the Agronomy Department, University of Florida. The procedures for the analysis were the same as those described in Chapter 4.

Results and Discussion

Means and variation for morphological characteristics evaluated in this experiment are presented in Table 5.1. Significant variability was observed for all the characteristics evaluated. While maximum canopy height of leaves occurred from 40 to 140 cm from the soil for the different populations, maximum height of inflorescences for the different populations occurred from 100 to 230 cm from the soil. Length of the inflorescences varied from 8.4 to 30.2 cm. and width of leaves varied from 0.6 to 1.4 cm. All plants flowered and the maximum number of heads per plant was 190.

The analysis of variance for the morphological characteristics is shown in Table 5.3. Differences among the populations were found for all the characteristics.

Table 5.1 Means and variation in morphological characteristics in five selected populations and two cultivars of *S. sphacelata.*

Characteristic	Mean ± st. dev.	Range
VH[+] (cm)	94 ± 17	40 to 140
RH (cm)	165 ± 21	100 to 230
VLW (cm)	0.97 ± 0.15	0.57 to 1.43
INFL (cm)	17.0 ± 3.6	8.4 to 30.2
HEAD (no.)	52.1 ± 30.3	1 to 190

[+]VH = vegetative plant height; RH = reproductive plant height; VLW = vegetative leaf width; INFL = inflorescence length; HEAD = number of heads.

Reproductive and vegetative plant heights were correlated (Table 5.2), as in the previous experiment (Chapter 4). Inflorescence length was correlated with reproductive height and number of heads was correlated with both vegetative and reproductive plant heights. The correlation of vegetative leaf width and inflorescence length was 0.31, similar to the previous experiment. The correlation of inflorescence length and head number was very low (r = 0.05) similar to the 0.028 obtained by van Wijk (1980).

Table 5.2 Correlation between morphological characteristics in five selected populations and two cultivars of *S. sphacelata.*

Morphological characteristic	VH[1]	RH	VLW	INFL
Vegetative height (VH)				
Reproductive height (RH)	0.60 [+]			
Vegetative leaf width (VLW)	0.12 [#]	0.14		
Inflorescence length (INFL)	0.19	0.36	0.31	
Number of heads / plant (HEAD)	0.40	0.38	- 0.02 [$]	0.05 [‡]

[+] all correlations have probabilities < 0.01 unless indicated
[#] P = 0.012; [$] P = 0.60; [‡] P = 0.29;

Table 5.3 Mean squares and (P > F) from the analysis of variance for morphological characteristics in five selected populations and two cultivars of *S. sphacelata*.

Source	df	VH*	RH	VLW	INFL	HEAD
Rep	3	0.668 (0.01)	0.491 (0.01)	0.103 (0.01)	37.550 (0.03)	7214.536 (0.01)
Pop	6	0.173 (0.01)	0.086 (0.03)	0.195 (0.01)	32.542 (0.02)	4739.357 (0.01)
Error	415	0.023	0.036	0.020	12.267	715.639

*VH = vegetative plant height (m); RH = reproductive plant height (m); VLW = vegetative leaf width (cm); INFL = inflorescence length (cm); HEAD (no.) = number of heads.

The comparisons of means for the seven populations are shown in Table 5.4. Splenda had a higher average canopy height than any other population with the exception of FROST Q1. The shortest population was FROST 3, which was only different from the above two and FROST 2. The source population AFRU had the tallest reproductive height, but only differed from FROST 3 and Solander. The shortest population was once again FROST 3. Selection for frost tolerance in Gainesville, therefore, had effect of reducing average canopy height of the second cycle and reproductive height of the third cycle.

Table 5.4 Means of morphological characteristics of five selected populations and two cultivars of *S. sphacelata*.

Population	VH[+]	RH	VLW	INFL	HEAD
	---------- cm ----------				no.
AFRU	93 c ‡	171 a	0.96 c	17.6 a	49.5 b
FROST 1	95 bc	163 abc	0.88 d	15.7 b	56.5 b
FROST 2	88 de	164 abc	1.01 b	17.6 a	49.7 b
FROST 3	87 e	160 c	0.95 c	17.0 ab	52.6 b
FROST Q1	99 ab	168 ab	0.98 bc	17.3 a	67.2 a
Solander	93 cd	162 bc	1.06 a	17.4 a	39.0 c
Splenda	102 a	164 abc	0.98 bc	16.5 ab	49.1 b
Min Sign Diff	5	8	0.05	1.4	9.1

[+] VH = vegetative plant height; RH = reproductive plant height; VLW = vegetative leaf width; INFL = inflorescence length; HEAD = number of heads.
‡ Means followed by the same lower case letter within a column do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Solander had the widest leaves of all populations (Table 5.4). This was also observed in the previous experiment (Chapter 4), where only the population selected for

width of leaves had similar leaf width as Solander. FROST 2 had the second widest leaves. FROST 1 had the most narrow leaves, followed by AFRU and FROST 3. A reduction in leaf width occurred in the first cycle of selection, followed by an increase in the second cycle and a decrease in the third cycle. Selection for frost tolerance, therefore, did not have a consistent effect on width of leaves.

The populations that showed the longest inflorescences were AFRU, FROST 2, FROST Q1 and Solander, but they only differed from FROST 1 (Table 5.4). As with leaf width, a reduction in inflorescence length occurred in the first cycle of selection for frost tolerance, followed by an increase in the second cycle. Inflorescence length was also not consistently affected by selection for frost tolerance.

Population FROST Q1 had a significantly higher number of heads than the other populations (Table 5.4). Solander had a significantly lower number of heads than all other populations. Populations AFRU and the three cycles of selection for frost tolerance at Gainesville had a similar number of heads. No explanation is apparent for the increase in head number observed in the FROST Q1 population.

Considering all the evaluated morphological characteristics, selection for frost tolerance in Gainesville seemed to have reduced the height of the plants while not affecting the other morphological characteristics. The vegetative plant height in the second and third cycles and the reproductive plant height in the third cycle were significantly reduced. However, selection for frost tolerance in Quincy significantly increased vegetative plant height and number of heads.

The analysis of variance for the close-spacing, sward-type experiment is shown in Table 5.5

Table 5.5 Mean squares and (P > F) from the analysis of variance for total, leaf and stem dry matter yields, leaf percentage and leaf:stem ratio (L/S) of five selected populations and two cultivars of *S. sphacelata*.

Source	df	Total DM	Leaf DM	Stem DM	Leaf %	L/S
Rep	2	4.358 (0.01)	0.144 (0.07)	3.080 (0.01)	68.460 (0.05)	0.032 (0.11)
Pop [+]	6	0.939 (0.03)	0.221 (0.01)	1.067 (0.11)	160.319 (0.01)	0.079 (0.01)
Error A (Rep*Pop)	12	0.277 (0.94)	0.030 (0.74)	0.227 (0.91)	19.497 (0.46)	0.013 (0.46)
Harvest	1	7.593 (0.01)	0.287 (0.02)	18.849 (0.01)	1754.205 (0.01)	0.639 (0.01)
Pop*Harvest	6	0.847 (0.34)	0.044 (0.46)	0.652 (0.31)	31.367 (0.20)	0.024 (0.14)
Error B	14	0.681	0.044	0.496	18.629	0.012
CV (%)		19.1	23.3	23.4	17.7	31.9

[+] P values derived from F = MS Pop / MS Error A

Differences among populations occurred for all agronomic characteristics with the exception of stem dry matter yield. Differences between harvests occurred for all characteristics, but there was no population by harvest interaction for any variable.

Over harvests, total dry matter production varied from 3430 to 5660 kg ha^{-1} (Table 5.6). Growth periods for these harvests were 56 d after seedling transplant for the first harvest and 48 d regrowth for the second harvest. Levels of production were similar to those obtained under grazing in Australia, where a variation of 3510 to 4790 kg ha^{-1} and 3400 to 5280 kg ha^{-1} were obtained in two experiments (Jones and Evans, 1989; Hacker and Evans, 1992). In the total of 104 d of growth in our experiment, the populations produced from 7750 to 9620 kg ha^{-1}.

Populations FROST 3 and FROST Q1 had the highest total dry matter yields, but they did not differ from AFRU, FROST 2 or Splenda (Table 5.6). There was a reduction in total dry matter production in the first cycle, but this was recovered with each successive selection cycle after that. The exceptional production of AFRU in the first harvest may have been responsible for the depression observed in the first cycle of selection. The production of AFRU in the first harvest was much higher than that in the second harvest, while the production of the three cycles of selection was similar in both harvests. The fact that the yields did not decrease with selection is an indication that variability was maintained over selection cycles and that no inbreeding depression occurred.

Although the very high yield of AFRU in the first harvest seems to be abnormal, FROST Q1 had a similar performance, and no statistical difference between FROST Q1 and AFRU occurred in any of the harvests or in the total of harvests (Table 5.6).

Comparing the standards, Splenda did not differ from Solander throughout the entire experimental period. The finding of no population by harvest interaction also supports a similar response of these selections over harvests.

Table 5.6 Individual harvest and seasonal total dry matter yield of five selected populations and two cultivars of *S. sphacelata*.

Population	13 July	30 Aug.	Total
	---------- kg ha^{-1} ----------		
AFRU	5590 a [‡]	3590 b	9180 ab
FROST 1	3860 b	3890 ab	7750 b
FROST 2	4370 b	3910 ab	8280 ab
FROST 3	4940 ab	4630 a	9580 a
FROST Q1	5660 a	3960 ab	9620 a
Solander	4420 b	3430 b	7850 b
Splenda	4430 b	3920 ab	8350 ab
Mean	4750 A	3900 B	8650

[‡] Means followed by the same lower case letter within a column or upper case letter within a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

The high production of AFRU and FROST Q1 in the first harvest was mainly due to high stem production (Table 5.7). Despite this, AFRU had the second highest leaf:stem ratio (Table 5.8). Cycles 1 and 2 of frost tolerance had the lowest leaf dry matter production over both harvests. There were no differences in leaf dry matter yield among AFRU, FROST 3, FROST Q1, Solander and Splenda. Over harvests, leaf dry matter yield varied from 380 to 1200 kg ha^{-1}. These levels were lower than the 1340 to 1760 kg ha^{-1} obtained in Australia (Hacker and Jones, 1992). Selection for frost tolerance had the

effect of decreasing leaf dry matter yield in the first two cycles, but this effect was nullified in the third cycle.

The relation of total and leaf dry matter is shown in Figure 5.2. In this figure, the low production of leaves in relation to the total production can be seen, as well as the small difference in production among the populations.

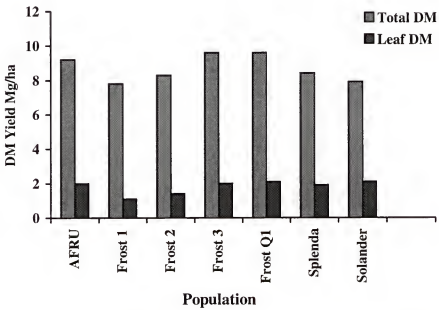

Figure 5.2 Total and leaf dry matter yield of five selected populations and two cultivars of *S. sphacelata.*

Populations AFRU and FROST Q1 also had a higher stem dry matter production than FROST 1 or FROST 2 in the first harvest and the same production as FROST 3 (Table 5.7). But in the second harvest, a different trend occurred. Population FROST 3 and Splenda had the highest stem production but were only different from Solander. In the total of harvests, stem production was higher for FROST 3 and FROST Q1, but only different from the first two frost selection cycles and Solander. Comparing the two standards, Solander and Splenda had similar leaf productions, while Splenda had higher

stem production. The higher stem production in Splenda was also observed in the previous experiment (Chapter 4). Over harvests, stem dry matter yield varied from 1610 to 4510 kg ha^{-1}.

Table 5.7 Individual harvest and seasonal leaf and stem dry matter yield of five selected populations and two cultivars of *S. sphacelata*.

	Leaf dry matter yield			Stem dry matter yield		
Population	**13 July**	**30 Aug.**	**Total**	**13 July**	**30 Aug.**	**Total**
	----------	----------	kg ha^{-1}	----------	----------	----------
AFRU	830 ab ‡	1090 ab	2020 a	4460 a	2050 ab	6510 ab
FROST 1	380 c	750 c	1130 b	3100 c	2450 ab	5550 bc
FROST 2	620 bc	750 c	1380 b	3310 bc	2150 ab	5460 bc
FROST 3	790 ab	1200 a	1980 a	3960 ab	2920 a	6870 a
FROST Q1	970 a	1120 ab	2080 a	4510 a	2550 ab	7070 a
Solander	1070 a	1020 ab	2090 a	3110 c	1610 b	4730 c
Splenda	950 ab	930 bc	1880 a	3340 bc	2670 a	6010 ab
Mean	810 B	980 A	1790	3680 X	2340 Y	6020

‡ Means followed by the same lower case letter within a column or upper case letter within a response variable in a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Leaf percentage of Solander was highest in both harvests, but it did not differ from Splenda in the first harvest or from AFRU in the second harvest (Table 5.8). Population FROST 1 had a lower leaf percentage in the first harvest and mean of harvests, but did not differ from FROST 2 in the mean of harvests. In the second harvest it only differed from AFRU and Solander.

Leaf percentages were very low and varied from 11.2 to 43.3%. These values are much lower than those obtained in Chapter 4 (41 to 63%) or in the work of Hacker

(1991a) (50 to 66%). It is believed that the reason for the low leaf percentages is the long harvest interval to which the plants were exposed. The growth period of Harvest 1 was 8 wk, while the second harvest interval was 7 wk. Leaf percentages were much lower in the first harvest (mean of 17.8%) than in the second harvest (mean of 30.9%). Van Wijk (1980) also obtained a reduction in leaf percentage from 40% at 6-wk regrowth to 24% at 9-wk regrowth in setaria. Since leaf percentages were low, leaf:stem ratios which varied from 0.22 to 0.58, were also lower than the ones reported in the literature. Hacker and Jones (1992) obtained leaf:stem ratios of 0.94 to 2.08 for four cultivars of setaria in Australia.

Agronomic data are relative to the non-weed fraction. The weed fraction was separated from the total plot production, because there was a wide variation in weed quantity in the different plots (3 to 120 g weed dry matter per m^2 plot). Furthermore, there was a large difference in dry matter percentages in the weed and non-weed fraction of the samples. Dry matter percentages in the leaves varied from 23 to 34.8% (mean of 27.3 ± 2.7%) and in the stems from 20.1 to 28.6% (mean of 24.3 ± 1.7%). However, dry matter percentages of weeds varied from 8.4 to 63.8% (mean of 26.1 ± 14.9%). The reason for this is believed to be due to the weed fraction being composed of many different species of weeds which were distinctive to different parts of the field. Therefore, due to the great variation in weed quantity per plot, and to the different weed dry matter percentages found in different plots, it was important to separate the weed fraction from the plot production. In experiments that report production data, if the weed fraction is present and not accounted for, there certainly will be errors associated with the yields reported.

Table 5.8 Individual harvest and seasonal leaf percentage, and seasonal leaf:stem ratio of five selected populations and two cultivars of *S. sphacelata*.

	Leaf percentage			L/S ratio
Population	**13 July**	**30 Aug.**	**Mean**	**Mean**
AFRU	17.2 b ‡	34.8 ab	26.0 b	0.37 b
FROST 1	11.2 c	24.1 c	17.6 c	0.22 c
FROST 2	15.7 b	26.7 bc	21.2 bc	0.28 bc
FROST 3	16.6 b	30.2 bc	23.4 b	0.32 bc
FROST Q1	17.3 b	30.8 bc	24.0 b	0.33 bc
Solander	25.6 a	43.3 a	34.4 a	0.58 a
Splenda	21.9 a	26.1 bc	24.0 b	0.32 bc
Mean	17.9 B	30.8 A	24.4	0.35

‡ Means followed by the same lower case letter within a column or upper case letter within a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

The probabilities of the calculated F obtained from the analysis of variance exceeding the Table F for CP and IVOMD in the leaves, stems and whole plants are shown in Table 5.9. There were no differences among the populations for any of these quality parameters. Differences between harvests were found for CP in the leaf, stem and whole-plant fraction and IVOMD in the leaf fraction. There was no interaction of harvest with population.

Over the two harvests, CP varied from 83 to 146 g kg^{-1} in the leaves and from 34 to 72 g kg^{-1} in the stems (Table 5.10). As reported in the literature, CP in the leaves was appreciably higher than in the stems (Zanetti et al., 1992). Populations FROST 1 and FROST 2 had the highest CP in the leaves in the first harvest, but they only differed from Solander and Splenda.

Table 5.9 Probabilities of calculated F > table F for crude protein (CP) and in vitro organic matter digestibility (IVOMD) in leaves, stems and whole plant (WP) of five selected populations and two cultivars of *S. sphacelata*.

	CP			IVOMD		
Source	**Leaf**	**Stem**	**WP**	**Leaf**	**Stem**	**WP**
	---------- g kg^{-1} ----------					
Rep	0.0005	0.1517	0.0532	0.1603	0.0570	0.0374
Pop	0.1771	0.5006	0.2343	0.5505	0.5613	0.6162
Rep*Pop	0.7361	0.7841	0.7230	0.4774	0.4184	0.4978
Harvest	0.0001	0.0001	0.0001	0.0006	0.2072	0.8679
Pop*Harvest	0.0750	0.5727	0.2676	0.4837	0.4586	0.4836

In the second harvest, AFRU, FROST 1 and Splenda had the highest CP in the leaves and they only differed from FROST 2 and FROST 3. No differences were found in the mean of harvests. In the stem, there were only differences for Harvest 2. Populations AFRU and Splenda had the highest CP values, but they only differed from FROST 2, FROST 3 and FROST Q1. Crude protein values in the leaves and in the stems were lower in the first harvest than in the second harvest. The reason may be the longer growing period (8 wk) of the first harvest than the harvest interval of the second period (7 wk).

Mean CP in the whole plant was 49 g kg^{-1} in the first harvest and 86 g kg^{-1} in the second harvest. Singh and Pradhan (1995) obtained a decrease in CP from 95 to 63 g kg^{-1} when harvested at 40 and 60 d of regrowth, respectively.

Across harvests, IVOMD varied from 400 to 522 g kg^{-1} in the leaves and from 402 to 464 g kg^{-1} in the stems (Table 5.11). The range in IVOMD values in the leaves were, therefore, larger than in the stems. There were differences in IVOMD for the first harvest in the leaves. Population FROST 2 had the highest IVOMD but it only differed

from Splenda. FROST 2 in the first harvest had the only digestibility value above 500 g kg^{-1}.

Table 5.10 Individual harvest and seasonal crude protein in leaves and stems of five selected populations and two cultivars of *S. sphacelata*.

	Leaf			Stem		
Population	**13 July**	**30 Aug.**	**Mean**	**13 July**	**30 Aug.**	**Mean**
	---------- g kg^{-1} ----------					
AFRU	102 ab ‡	146 a	124	40	70 a	55
FROST 1	106 a	145 a	126	43	65 ab	54
FROST 2	113 a	123 b	118	41	57 b	49
FROST 3	97 abc	125 b	111	38	59 b	49
FROST Q1	97 abc	132 ab	114	36	58 b	47
Solander	83 c	139 ab	111	34	67 ab	51
Splenda	88 bc	145 a	116	35	72 a	54
Mean	98 B	136 A	117	38 X	64 Y	51

‡ Means followed by the same lower case letter within a column or upper case letter within a response variable in a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Values of IVOMD were most frequently higher in the leaves than in the stems in the first harvest and the mean of harvests, but were inconsistent in the second harvest. Hacker et al. (1974) obtained values of 2 to 3 units higher digestibility in the leaves than in the stems. This is in contrast to the data presented in Chapter 4, where stem IVOMD was higher than leaf IVOMD. The quality of setaria could be improved by improving leaf digestibility, as stated by Hacker (1971).

There were differences in whole-plant CP or IVOMD means only in the second harvest CP (Table 5.12). At this harvest, AFRU and Solander had the highest levels and they

differed from all populations except Splenda. No differences were observed in the mean of harvests for CP or IVOMD. Whole-plant CP varied from 46 to 96 g kg^{-1} and whole-plant IVOMD from 410 to 464 g kg^{-1}. Whole-plant CP and IVOMD obtained in this experiment were lower than those reported in Chapter 4. However, this was expected because the growing intervals in this experiment were longer. A longer growing interval also permitted more flowering, thus a decrease in these concentrations as reported to occur in setaria for CP (Perera et al., 1990; Singh and Pradhan, 1995) and IVOMD (Hacker and Minson, 1972; van Wijk, 1980; Moss and Murray, 1992)

Table 5.11 Individual harvest and seasonal in vitro organic matter digestibility in leaves and stems of five selected populations and two cultivars of *S. sphacelata*.

	Leaf			Stem		
Population	**13 July**	**30 Aug.**	**Mean**	**13 July**	**30 Aug.**	**Mean**
	---------- g kg^{-1} ----------					
AFRU	491 ab [+]	408	450	414	433	424
FROST 1	481 ab	451	466	402	429	416
FROST 2	522 a	416	469	438	428	433
FROST 3	479 ab	463	471	417	444	431
FROST Q1	488 ab	445	467	427	437	432
Solander	487 ab	449	468	422	454	438
Splenda	466 b	400	433	464	436	450
Mean	488 A	433 B	460	426	437	432
		NS [#]	NS	NS	NS	NS

[+] Means followed by the same lower case letter within a column or upper case letter within a response variable in a row do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

[#] Non significant

In Figures 5.3 and 5.4, the CP and IVOMD values of leaf, stem and whole plant for the populations can be seen graphically. Leaf CP (Figure 5.3) and IVOMD (Figure 5.4) were higher than in stems, and may be due to the low leaf production obtained in relation to stems, resulting in increased CP concentration in stems. Leaf IVOMD was lower than stems only in Splenda (Figure 5.4).

Based on these results, it is concluded that selection for frost tolerance in Setaria had no effect on the nutritive value of the forage in the leaf or stem fractions or in the whole plant.

Table 5.12 Individual harvest and seasonal crude protein (CP) and in vitro organic matter digestibility (IVOMD) in the whole plant of five selected populations and two cultivars of *S. sphacelata*.

	CP			IVOMD		
Population	**13 July**	**30 Aug.**	**Mean**	**13 July**	**30 Aug.**	**Mean**
	---------- g kg^{-1} ----------					
AFRU	51 [+]	96 a	74	427	426	426
FROST 1	50	83 bc	66	410	434	422
FROST 2	52	74 c	63	451	439	447
FROST 3	47	79 c	63	427	449	438
FROST Q1	47	80 bc	64	438	439	438
Solander	47	96 a	72	438	446	442
Splenda	46	90 ab	68	464	427	446
Mean	49 B	86 A	67	436	437	437
	NS [#]	-	NS	NS	NS	NS

[+] Means followed by the same lower case letter within a column or upper case letter within a response variable in a row do not differ significantly at the $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.
[#] Non significant

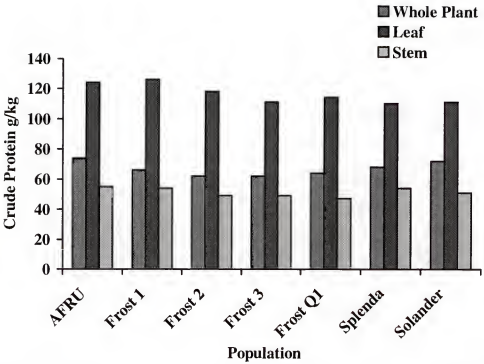

Figure 5.3 Crude protein concentration in the whole plant, leaves and stems of five selected populations and two cultivars of *S. sphacelata*.

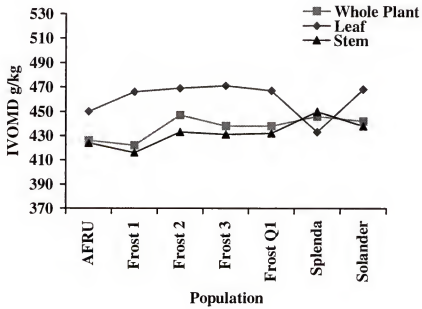

Figure 5.4 Whole-plant, leaf and stem in vitro organic matter digestibility (IVOMD) of five selected populations and two cultivars of *S. sphacelata*.

The monthly mean minimum winter temperatures in 1999-2000 were concentrated during the months of December to February, and the December 1999 mean minimum was below the 70–yr average (Figure 5.1). Not considering 1 d with a minimum temperature below 0°C on 4 Nov. 1999, there were 18 d with minimum temperatures below 0°C during the 84 d period from 1 Dec. 1999 to 22 Feb. 2000. In the winter of 1997-1998, with the exception of 1 d with minimum temperature below 0°C on 17 Nov. 1997, there were only 11 d with minimum temperatures below 0°C in the period of 100 days, from 6 Dec. 1997 to 15 Mar. 1998. In the winter of 1998-1999, there were 19 days with minimum temperatures below 0°C in the period of 92 d, from 16 Dec. 1998 to 17 Mar. 1999. The fact that the very cold days in the winter of 1999-2000 were concentrated in a short period of time appeared to have a strong effect on the survival of the populations that were planted on 6 Oct. 1999. The original populations at Gainesville, from which the populations were selected, had a 19% survival rate, while the three selections from Gainesville had from 36 to 40% survival (Table 5.13). Population FROST Q1 had a lower survival rate than the populations selected at Gainesville. Splenda was the Australian population with the best survival (11%). Thompson Falls which is from the high altitude Aberdares Region of Kenya, and is known to exhibit genes for frost tolerance did not survive the winter of 1999-2000 in Florida (Table 5.13). Hacker (1972) found a negative relationship between vigor and frost tolerance in the frost-tolerant populations and a poor stand persistence of frost tolerant lines due to a susceptibility to short cutting height. In our experiment, Thompson Falls was less vigorous than other populations, but had not suffered defoliation. The survival of Solander, a cultivar that also has from 11 to 34% genes from frost-tolerant accessions in

its ancestry (Oram, 1986), also showed poor survival after the winter of 1999-2000 in Florida. This suggests that these high altitude accessions show more tolerance to frost in the conditions of southeast Australia, where radiation frosts occur. Temperature inversion with clear skies and calm nights with winds below 10 mph characterize radiation frosts. In Gainesville, radiation frost may occur, but harder frosts and freezes also occur with temperatures as low as -8°C recorded on 6 Jan. 1999. The higher survival of the base populations and its selections, is an indication that the base population made up of the cultivars Narok, Kazungula and Solander, and roadside plants may have genes for frost tolerance for the conditions at Gainesville that are not exhibited by the high altitude accessions in Kenya. It also suggests that genes that will not express in one environment may express in another one when triggered to do so.

Selection for frost tolerance was effective in increasing the survival rates after the exposure to frosts. In the sward-type experiment (30-cm center), each cycle of selection increased the survival rate (Table 5.13). Although there was no difference among the rates of the three cycles, only the rates in Cycle 3 were different than from the original population. The survival rate of 6% in the original population was increased to 14% in the first cycle, to 24 and 27% in the two consecutive cycles, respectively. Survival rates in the wide-spacing experiment (90-cm center) were much higher than in the close-spacing experiment. The presence of more bare soil in the widely spaced experiment may have offered microclimate temperature modification from heat radiating from the bare soil. As in the close-spacing experiment, the three FROST populations had the highest survival rates and they were not different from each other. However, only FROST 1 and

FROST 3 were superior to the original population. FROST 1 had the highest rate, and it was double that of the original population.

The survival rates of FROST Q1 were lower than any of the FROST cycles selected in Gainesville, and they did not differ from the rates of the original population. This was unexpected, since FROST Q1 is a selection from a population similar to FROST 2 that was planted in Quincy. Selection in Quincy was not as effective as selection in Gainesville. There were 3 d with temperature below 0°C in December 1998 in Quincy and 4 d in February 1999. Unfortunately, temperature data for January 1999 in Quincy were not available. Both the heritability (Table 3.10) and expected gain from selection (Table 3.11) calculated for Quincy were lower than for Gainesville. The winter of 1999-2000 that killed many of the setaria plants was effective in the screening for frost susceptible and frost tolerant material and further research in this field is promising.

Table 5.13 Percentage *S. sphacelata* plant survival on 30 Mar. 2000 after the winter months 1999 to 2000 at Gainesville, Florida.

	Field spacing		
Population	**30-cm center**	**90-cm center**	**Mean** [#]
	% plant survival after winter 1999 to 2000		
AFRU	6 bc [+]	31 cd	19
FROST 1	14 abc	63 a	39
FROST 2	24 ab	48 abc	36
FROST 3	27 a	52 ab	40
FROST Q1	11 abc	39 bc	26
Splenda	6 bc	16 de	11
Solander	1 c	8 e	5
Thompson Falls	0 c	2 e	1

[+] Means followed by the same lower case letter within a column do not differ significantly at $P > 0.05$, according to the Waller-Duncan multiple comparison procedure.

Summary and Conclusions

Three populations of *S. sphacelata* selected in Gainesville and one selected in Quincy, Fl, for frost tolerance, were compared to the original population and to two commercial standards in two experiments.

In one experiment, populations were compared morphologically. Selection for frost tolerance in Gainesville had the effect of reducing maximum height of the leaves in the second cycle and the maximum height of the inflorescences in the third cycle. It did not have a consistent effect on the width of leaves or length of the inflorescences. Number of heads was also not affected. However, selection for frost tolerance in Quincy increased maximum height of the leaves and the number of heads.

In a second experiment, populations were compared agronomically. Selection for frost tolerance in Gainesville had the effect of decreasing leaf dry matter yield in the first two cycles, but this effect was nullified in the third cycle. It did not have an effect on total or stem dry matter production or on leaf:stem ratio.

The survival in a concentrated cold winter 1999-2000 in Gainesville was lower overall for all populations. The original Gainesville population and the selections had a much higher survival rate than the Australian populations known to carry genes for frost tolerance. This is an indication that the base population made up of the cultivars Narok, Kazungula and Solander and roadside plants may have genes for frost tolerance for the conditions at Gainesville not exhibited by the high altitude accessions in Kenya.

All cycles of selection had a higher survival rate after frosts than the original population. This indicates that selection for frost tolerance in Gainesville was successful. The winter of 1999-2000 that killed many of the setaria plants was effective in providing

additional screening for frost and freezing tolerance and further research under Florida conditions appears warranted.

CHAPTER 6
GENERAL CONCLUSIONS

The purpose of this chapter is to summarize findings and practical implications of the experiments decribed in Chapters 3 through 5. These experiments were conducted to study the breeding potential and adaptation of the tropical forage grass *S. sphacelata* to Florida conditions. The work was undertaken because there is a lack of adapted warm-season grasses for these conditions. In the period of 1997 to 1999, seven populations were developed, five at Agronomy Forage Research Unit, north of Gainesville, one at the Range Cattle REC in Ona, Florida, and one at the North Florida REC, Quincy, Florida. In 1999 and 2000, these populations were compared in four experiments at the AFRU. The objectives were to evaluate the modifications of half-sib family selection methodology to reduce selection cycle interval in *S. sphacelata* breeding programs, to produce advanced populations of the species selected for morphological and physiological traits of agronomic value, and to evaluate the effects of selection for morphological and physiological traits on agronomic performance in a common environment.

The development of the populations was described in Chapter 3. The initial field was established in 1996 with a mixture of seeds of Narok, Kazungula and Solander, and roadside plants collected at the Range Cattle REC. A population selected for vigor from the initial field was variable for the morphological characteristics reproductive and vegetative plant heights, flag and vegetative leaf widths, inflorescence length and number of heads. Heritabilities ranged from 0.17 to 0.86, the highest being for flag leaf width.

The use of the modification of half-sib family selection methodology with some restrictions suggested by Burton (1982), permitted intercrossing of selected plants in the greenhouse, thus eliminating the need to plant isolation blocks of the selected plants in the field. This methodology was found to be useful for Setaria breeding, because it was possible to complete one cycle of selection per year. Also, with this methodology, variability was maintained in the selected cycle and inbreeding was avoided.

Two cycles of selection were conducted for increased number of heads. Despite heritability being 0.4, this character did not respond well to selection. A possible explanation for the lack of response is that our experimental protocol may not have allowed us to separate the genetic variance component from the environmental variance component of the total phenotypic variance. The means of the first cycle of selection for head number were much lower than the second cycle, but both were lower than the initial population selected for vigor. It was speculated that the soils where the two selections were planted were less fertile than where the initial population was planted. The soil of the initial populations was an inceptisol, while that of the selections was a spodosol. The latter is characterized as having a lower fertility and being seasonally waterlogged.

One cycle of selection for increased leaf width was conducted. A heritability of 0.69 was obtained and a gain of 16% in the width of leaves over the initial population was predicted.

In Ona, the setaria population was periodically mob-grazed during October 1997 to March 1998. In the summer of 1998, a severe attack of chinch bug (*Blissus leucopterus*) decimated the plots. Open-pollinated seeds of the few surviving plants were used as the population selected under grazing.

Three cycles of selection were conducted for early spring growth after frost and freezes in Gainesville with the second cycle also planted at Quincy, where another cycle was selected. Heritability estimates for the first two cycles in Gainesville and the cycle in Quincy ranged from 0.21 to 0.22 on an individual plant basis and from 0.35 to 0.41 on a family basis. Predicted gains ranged from 10 to 14% in tolerance to frost damage over the initial preceeding cycle.

The comparison of the first four populations was described in Chapter 4. Populations were compared to the original populations from the AFRU and from Ona. The standards included were two commercial cultivars Splenda and Solander, and two frost tolerant introductions (Fenwicks and Thompson Falls). Two experiments were conducted: one wide-spaced (90-cm centers) and the other close-spaced (30-cm centers).

The results showed that selection for leaf width increased the width of leaves, the percentage of leaves, leaf:stem ratio, and whole-plant CP concentration. However, it decreased stem and total dry matter yield and did not affect leaf dry matter yield or IVOMD.

Selection for head number did not increase the number of heads and decreased plant height, leaf width and inflorescence length in one or both cycles. No increase in seed production was obtained from the selection for increased number of heads. Selection for number of heads also decreased total and stem dry matter yields, and leaf dry matter yield, leaf percentage and leaf:stem ratio in the first cycle. The CP and IVOMD were not affected by this selection.

Selection for regrowth after grazing resulted in reduced plant height and inflorescence length and reduced seed production. The effects on dry matter yield and

nutritive value were not determined because of lack of seeds to include this population in the sward experiment.

The comparison of the frost-tolerant populations was described in Chapter 5. Three populations of *S. sphacelata* selected in Gainesville and one selected in Quincy, Fl, for frost tolerance, were compared at the AFRU to the original population and to Solander and Splenda in two experiments: a wide-spaced and a close-spaced experiment.

Results showed that selection for frost tolerance in Gainesville had the effect of reducing average height of the canopy in the second cycle and the maximum height of the inflorescences in the third cycle. It did not have a consistent effect on the width of leaves or length of the inflorescences. Number of heads was also not affected. However, selection for frost tolerance in Quincy increased average maximum height of the canopy and the number of heads. Selection for frost tolerance in Gainesville had the effect of decreasing leaf dry matter yield in the first two cycles, but this effect was nullified in the third cycle. It did not have an effect on total or stem dry matter production or on leaf:stem ratio.

Selection for frost and freezing tolerance in Gainesville was successful, since all cycles of selection had a higher survival rate after the winter of 1999-2000 than the original population. The original population and the selections also had a higher survival rate than the Australian populations known to carry genes for frost tolerance. This is an indication that the base population made up of the cultivars Narok, Kazungula and Solander, and roadside plants may have genes for winter survival under the conditions at Gainesville not exhibited by the high altitude accessions in Kenya.

The main practical finding from these experiments is that *S. sphacelata* proved to be a promising plant for Florida conditions. The germplasm was plastic enough that selection and breeding for morphological and agronomic traits were successful, particularly for improvement of leaf width. Dry matter production levels were comparable to other tropical pasture grasses and CP concentration levels were higher. Levels of IVOMD, however, were lower than reported for Setaria grown in other locations and lower than for some other tropical grasses of similar age. The detached culms technique for intermating selected plants proved to be an adequate methodology for developing improved populations in Setaria.

The winter of 1999-2000 that killed many of the setaria plants was effective in providing additional screening for frost and freezing susceptible tolerance and further research with this species for Florida conditions appears warranted. Surviving plants have been transplanted to a polycross nursery, and will be exposed to another winter (2000-2001) in the field and selection for frost-freezes survival will be made before selected plants are intercrossed and a new cycle developed. Recommendations outlined in Chapter 4 regarding nutritive value and pest resistance research must also be applied to any additional populations selected for winter survival.

APPENDIX

Table A1 Climatological data for the experimental period (October 1996 to September 2000) at Gainesville, Florida.

		Temperature °C			Rainfall	No. days	Number of days below temp:		
Year	**Month**	**Min**	**Max**	**Mean**	**mm**	**drought**	**0°C**	**4°C**	**12°C**
1996	Oct	2	32	21	133	1	0	1	9
	Nov	- 1	31	17	27	24	1	7	16
	Dec	- 5	28	14	140	1	4	9	22
1997	Jan	- 7	22	14	72	0	5	10	24
	Feb	0	31	16	36	11	1	4	19
	Mar	2	32	21	87	15	0	1	8
	Apr	3	31	19	182	17	0	2	16
	May	6	33	23	83	3	0	0	5
	Jun	16	34	25	194	0	0	0	0
	July	18	38	27	216	0	0	0	0
	Aug	14	36	27	155	0	0	0	0
	Sept	14	37	26	47	26	0	0	0
	Oct	4	33	21	136	18	0	1	7
	Nov	- 1	29	16	118	0	1	4	20
	Dec	- 4	27	13	259	0	3	12	23
1998	Jan	-4	27	14	102	0	3	13	20
	Feb	0	27	14	358	0	1	9	21
	Mar	- 3	31	15	99	0	4	12	19
	Apr	4	32	20	6	23	0	1	12
	May	12	37	25	73	27	0	0	1

Table A1 Continued

	Jun	18	39	29	45	20	0	0	0
	July	21	38	29	212	6	0	0	0
	Aug	19	37	28	181	0	0	0	0
	Sept	16	36	26	237	0	0	0	0
	Oct	8	33	23	89	9	0	0	7
	Nov	3	31	20	10	27	0	2	8
	Dec	- 3	29	17	14	26	4	6	18
1999	Jan	- 8	28	15	128	11	6	11	23
	Feb	- 3	28	15	54	5	6	12	19
	Mar	- 2	29	16	54	21	3	10	29
	Apr	4	35	22	27	26	0	1	9
	May	9	33	23	101	18	0	0	5
	Jun	18	37	26	184	8	0	0	0
	July	20	38	28	134	0	0	0	0
	Aug	20	39	28	186	3	0	0	0
	Sept	13	36	26	209	5	0	0	0
	Oct	2	32	22	15	4	0	2	8
	Nov	- 1	30	18	64	15	1	5	19
	Dec	- 5	28	13	32	20	7	13	23
2000	Jan	- 6	28	13	90	21	4	13	27
	Feb	- 2	28	14	18	10	7	12	26
	Mar	2	31	19	76	25	0	1	21
	Apr	2	32	19	20	22	0	4	16
	May	9	38	25	3	31	0	0	1
	Jun	15	39	27	170	18	0	0	0

Source: Ben Whitty and Richard Hill, Weather Office, Agronomy Department, IFAS, University of Florida, Gainesville, Florida, 32511.

Table A2 Climatological data for 1998 and 57-yr average at Ona, Florida.

	Air temp (°C)		Rainfall (mm)	
Month	**Min**	**Max**	**1998**	**57-yr avg.**
January	12.8	22.8	110	57
February	11.1	22.2	244	67
March	12.8	23.9	313	83
April	15.6	27.8	10	63
May	17.7	31.1	55	96
June	21.7	34.4	153	218
July	22.8	33.3	192	218
August	22.8	33.9	196	204
September	22.8	31.7	264	182
October	16.7	30.6	74	80
November	16.7	28.3	105	50
December	14.4	26.1	19	49
Total	-	-	1735	1367

Source: Kalmbacher (1999).

Table A3 Climatological data for the experimental period (October 1998 to April 1999) at Quincy, Florida.

	Air temp (°C)		Days below temp (°C)			Rainfall
Month	**Min**	**Max**	**0**	**4.4**	**10**	**(mm)**
Oct 1998	6.7	30	0	0	7	7
Nov 1998	3.9	28.9	0	1	9	19
Dec 1998	- 0.5	27.2	3	5	18	45
Jan 1999 *	-	-	-	-	-	-
Feb 1999	- 1.7	26.7	4	9	23	58
Mar 1999	- 2.2	27.2	1	6	25	71
Apr 1999	3.3	32.8	0	1	10	17

* Data not available

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BIOGRAPHICAL SKETCH

The daughter of German and Croatian immigrants, Liana Jank was born in São Paulo, Brazil, on 21 June 1956. She became interested in agronomy early in life due to numerous visits to a farm managed by her much older brother, also an agronomist. She studied Agriculture at the "Escola Superior de Agricultura 'Luiz de Queiróz'" of the University of São Paulo at Piracicaba, São Paulo, from 1975 to 1979. Since her third year at the University she became interested in forages, but only managed to pursue her dream of studying abroad for her masters in forage management in 1980. She received a scholarship from the American Chamber of Commerce in São Paulo and Rio de Janeiro and pursued her M.S. at the University of Florida under the guidance of Dr. G.O. Mott.

On October 1982, she was hired by EMBRAPA to work at the National Center for Beef Cattle Research (CNPGC) at Campo Grande, MS, Brazil. She works with evaluation, breeding and release of cultivars of *Panicum maximum.*

In January 1997 she initiated her Ph.D. program at the University of Florida with a scholarship from EMBRAPA. She is studying breeding of *Setaria sphacelata* under the guidance of Dr. K.H. Quesenberry. When she returns to Brazil, she will continue with her former job.

She is married to Silvano Calixto and has three beautiful children: Carina age 11, André age 10 and Angela age 9.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the Degree of Doctor of Philosophy.

Kenneth H. Quesenberry, Chair
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the Degree of Doctor of Philosophy.

Paul Lyrene
Professor of Horticultural Sciences

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the Degree of Doctor of Philosophy.

Paul Mislevy
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the Degree of Doctor of Philosophy.

David S. Wofford
Professor of Agronomy

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the Degree of Doctor of Philosophy.

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This dissertation was submitted to the Graduate Faculty of the College of Agricultural and Life Sciences and to the Graduate School and was accepted as partial fulfillment of the requirements for the Degree of Doctor of Philosophy.

May 2001

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